APPLIED MYCOLOGY AND BACTERIOLOGY

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FOREWORD

IT is obviously not possible to compress into one small volume a really adequate account of fungi and bacteria, together with a description of their applications to human affairs, a guide to laboratory technique, and a bibliography. Nevertheless some such attempt seems desirable. It is hoped that the following brief survey of the field of mycology and bacteriology will be of some service to biologists and chemists in co-ordinating their studies with those of other workers, and in indicating the scope and methods of economic microbiology.

The industrial chemist, in particular, is expected to cope with any problem, from plumbing to entomology. He would often like to examine in more detail some micro-organism that is affecting his work, but is at a loss how to begin; and it is a regrettable fact that the early training of a chemist seldom includes even the most elementary biology, although the converse is not the case. If he attempts to extract the details he requires from textbooks chosen at random among the extensive literature available, he is likely to be appalled at the enormous variety of organisms, the multiplicity of staining methods, and the technical jargon of books intended for those with a biological training. An even worse danger is that he should be over-confident, and lend the support of his learning to statements unacceptable to the expert.

This volume is intended only as an elementary review of essentials, and as an introduction and supplement to the fuller literature quoted. Medical microbiology has been barely touched on, and admittedly deserves a larger space than it receives here. The authors have endeavoured not to waste space in repeating material that may be found in any of the standard textbooks; readers wishing to see illustrations of incubators and other laboratory equipment are referred to the makers' catalogues. The bibliography has been kept small, and is limited for the most part to literature that is readily available and in which further references will be found. As

regards names of bacteria, Bergey's system has been followed except that common species like *Escherichia* (*Bacterium*) *coli* are sometimes referred to by the older and more familiar names.

The problem has been not what to put in, but what to leave out, and every reader will find some omissions that he will consider inexcusable. Errors may have been included, and undoubtedly many important references have been missed. The authors would be grateful for details of such errors and omissions.

The authors are deeply indebted to the following friends for advice in connection with this book:

Professor F. T. Brooks and Dr. R. St. John-Brooks (general criticism); Dr. L. R. Bishop (Chapter X.); Dr. W. C. V. Brothwood (Chapter XII.); Mr. W. C. Moore (Chapter XIII.); Dr. B. B. Mundkur (Chapters II. and XIII.); Dr. R. G. Tomkins (Chapter IX.); Dr. T. K. Walker (Chapters VII. and X.).

L. D. G.
R. B.

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A second printing has afforded the opportunity of revising the text and references, and also of adding to the illustrations.

For permission to publish Plates 2 and 4 the authors are indebted to Mr. T. McLachlan, in whose laboratory these photographs were taken.

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BACTERIA ON A MONUMENT.

Part of a central obelisk of the Pasteur Memorial at Strasbourg. The studies represented on this face include fermentation (1857), spontaneous generation (1860), and silkworm disease (1865). Similar panels on the remaining faces show other aspects of Pasteur's work—rabies, anthrax, etc. At the base of the obelisk is a chain of crystals representing Pasteur's earlier work on stereo-chemistry.

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PART ONE

CHAPTER I

INTRODUCTORY

THE organisms included under the names of fungi and bacteria affect our lives closely. The normal decomposition of organic matter is principally due to them, and without their aid the world would be strewn to an embarrassing extent with unrotted vegetation and undecayed animal corpses. They play an essential part in plant nutrition, whilst individual types are utilised by us in the preparation of many food products and for an increasing number of industrial processes.

On the other hand, we suffer considerably if the activities of micro-organisms are uncontrolled. "Perishable" substances become damaged or completely ruined, whilst our crops and even our bodies and those of our domestic animals are subject to diseases caused by them.

It is therefore of importance not only to the scientific worker, but also to the agriculturist, the industrialist, the housewife, and the man in the street, to know something more of micro-organisms than is at present included in a general education.

The best way to acquire an enthusiasm for the subject is to read one of the many excellent biographies (*e.g.*, Valery-Radot, 1919) of the famous Frenchman, Louis Pasteur. Few men can claim to have stimulated the advance of applied science in so many directions as did Pasteur (1822-1895). Originally trained as a chemist, he carried out a well-known piece of research on the stereo-isomerism of racemic acid, during which he observed that certain green moulds (*Penicillium* spp.) utilised *d*-tartaric acid in preference to *l*-tartaric. His interest thus aroused by the chemical activities of micro-organisms, he turned to the study of alcoholic, lactic, and butyric fermentations, which for the first time were shown to be due to living organisms. He then laid the bogey of "spontaneous

generation," and carried out pioneer work on " diseases " of vinegar, wines and beers, and diseases of silkworms. Lister's antiseptic system that revolutionised surgery arose directly from this earlier work of Pasteur, and Pasteur's own later work laid the foundations of the study and treatment of infectious diseases. Following up the principle of Jenner's vaccine treatment, he devised methods of inoculation against anthrax and rabies.

It is perhaps not surprising that microbiological studies concerned with the alleviation of human suffering took precedence, and that the development of bacteriology since 1870 has been largely confined to the study of pathogenic organisms. In fact, the word Bacteriology is still associated in the public mind with hospitals and disease " germs." Nevertheless, the pathogenic bacteria represent a small minority, and the study of other types, with a view to their utilisation or suppression, offers a vast field for research. It is probable that even now the good effects of micro-organisms far outweigh the harm they do, and as our knowledge extends it will be found increasingly possible to make use of the harmless types and to keep the harmful types under strict control.

The *Fungi*—with the exception of the yeasts, which have much in common with the bacteria—were a subject of study long before the bacteria. By reason of their larger size, they did not have to wait on the progress of microscopy. But it was not until about 1845 that certain of the more minute fungi were realised to be the cause of severe crop diseases. Fungi still remain to some extent the prerogative of the botanist, and just as bacteriology is often taken to mean medical bacteriology, so mycology (*i.e.*, the study of fungi) often implies plant pathology. Yet later studies have shown that the activities of fungi and bacteria are closely interwoven; both are active as agents in producing soil fertility, fermentation, and disease in plants and animals.

As a convenient term to cover the study of bacteria and of the more microscopic fungi (which include yeasts) the word Microbiology has of late years come into use; this in its industrial aspects covers the same ground as the terms Technical Mycology and Industrial Bacteriology. For textbooks of a general nature reference may be made to the list of titles at the end of this chapter.

It will be seen that the chemist, the botanist, and the pathologist have all played a part in developing our knowledge of micro-organisms, and a sympathetic co-operation by these three classes offers the best hope of further advances in such knowledge.

It is very desirable that the worker in applied microbiology should possess some knowledge of other forms of life than bacteria and fungi. He should be able to recognise the presence of algæ or protozoa in liquids he is examining for bacteria, and to detect mites if they occur as an infection of his fungal cultures and food products. A knowledge of plant and animal structures is often of considerable assistance in examining material under the microscope. Such a warning against too narrow specialisation is probably unnecessary, but it is given here, since the limits of the present volume preclude anything beyond a brief sketch of the fungi and bacteria.

Classification.

The primary object of classification is to secure an orderly arrangement of an otherwise unwieldy mass of material. The terms used should be in some universal language—since science is international—and the classification should as far as possible indicate the relationships considered to exist among the various forms—*i.e.*, it should be a " natural " classification.

What constitutes a basic unit of classification, or *species*, is very difficult to define, especially in the case of organisms which have a simple life history. In fact, so difficult is it to formulate a satisfactory definition that the following has been put forward: " A species is that which has been accepted as a species by a competent systematist "! However, in practice the limit of the species is fairly well appreciated, though some workers tend to segregate into species forms that other workers regard as merely varieties of one species.

Each organism is given a scientific name, usually more or less based on Latin or Greek roots, according to the binomial system introduced by Linnæus. Thus a certain common mould fungus is called *Cladosporium herbarum*; here *Cladosporium* is the *genus*, which is subdivided into a number of *species*, of which *C. herbarum* is one.

Practical Importance of Identification.

In considering the steps necessary to check some destructive manifestation of micro-organisms, the practical man is apt to say: "Why on earth waste time in identifying the organism? All I want to do is to suppress it." But the time spent in identification is usually—though not always—well spent. Even an approximate identification at once places the worker in touch with all the important information in the literature concerning this organism, or (showing the advantage of a "natural" classification) related organisms of similar habits. Such information is frequently of value in suggesting a method of control. Conversely, research done on any particular organism is practically valueless to later workers unless the organism is accurately identified. As an example of this one may quote the vast amount of chemical work that has been done on "*Penicillium glaucum*," a term that has been used in the past to mean practically any species of green *Penicillium*. Incidentally, such work is of little value unless precautions are taken, *and recorded*, to ensure that the work was started and completed under pure culture conditions—*i.e.*, in the absence of other organisms.

The best plan of specifying the organism used in any published work is to obtain it from, or deposit it with, one of the standard collections which are maintained. In England there now exists the National Collection of Type Cultures (Medical Research Council) at the Lister Institute, Chelsea, whence pure cultures of bacteria and fungi can be obtained at a nominal fee. A still larger collection of fungal cultures exists at the Centraalbureau voor Schimmelcultures, Baarn, Holland.

Systematic Position of Fungi and Bacteria.

The old distinction between plants and animals is firmly rooted in our minds, but in reality no sharp dividing line exists. In general, of course, animals move and plants are stationary; animals require complex food, whilst green plants utilise the sun's energy to build up proteins, carbohydrates, and fats from inorganic sources; best distinction of all, animals ingest solid food by means of a gullet, whereas plants must absorb their food in solution through a cell wall.

Among the simpler organisms, however, exceptions can be found to all these criteria. Many forms obviously related to green plants have lost their chlorophyll, and in consequence require organic food material; certain Myxomycetes (which are classed with plants) differ very little from the simpler Protozoa (which are classed with animals); whilst the bacteria show so little structural detail that it is difficult to say what their systematic position should be.

With these reservations, the following scheme may be given as a rough guide to the position of bacteria and fungi in the scheme of living organisms:

ANIMALS	Single celled	*Protozoa.*
	Many celled	*Metazoa* (including Mammals, Insects, Fishes, etc.).
PLANTS	Seed plants	*Phanerogams* (including Flowering Plants, Conifers, etc.).
	Seedless plants.. *Cryptogams*	Pteridophytes (Ferns, etc.).
		Bryophytes (Mosses and Liverworts).
		Thallophytes: Algæ, Fungi — Combined as Lichens.
		Bacteria.
		? Viruses.

REFERENCES

GILTNER, W. (1928): General Microbiology. London.

HENRICI, A. T. (1930): Molds, Yeasts, and Actinomycetes. New York.

LAFAR, F. (1898-1910): Technical Mycology. Translated C. Salter. London.

MARSHALL, C. E. (1921): Microbiology. London.

NICOL, H. (1939): Microbes by the Million. (Penguin Sixpenny.)

PARTRIDGE, W. (1938): Aids to Bacteriology. London.

SALLE, A. J. (1939): Fundamental Principles of Bacteriology. New York.

VALERY-RADOT, R. (1919): Life of Pasteur. London.

CHAPTER II

THE FUNGI

THE vast group Fungi cannot be described in a few pages, and the present chapter is intended merely as a précis, to be used in conjunction with more specialised textbooks.

The active part of a fungus usually consists of a system of filaments of microscopic thickness—the *hyphæ*—known collectively as the *mycelium*. This may form a compact mass of definite form, as in the familiar " mushrooms," or it may be simply a loose cottony mass ramifying in or on the substratum. In certain instances the hyphæ may be aggregated loosely into rope-like strands (*rhizomorphs*), or they may unite to form hard compact masses (*sclerotia*) capable of acting as a resting stage.

The mycelium possesses no chlorophyll, and to secure growth it must absorb suitable organic nutrient material in soluble form. Within the bounding cell wall are contents similar to those found in cells of higher plants—protoplasm, nuclei, and reserve food substances. In most groups of fungi the hyphæ are divided at intervals by cross-walls, whilst in other groups these occur but rarely. Fresh mycelium arises by the growth of existing mycelium, or by the germination of a structure known as a *spore* which is usually capable of undergoing a resting stage; reproduction and dissemination are ensured by the production of a fresh supply of spores later in the life history of the organism.

Spores may be of two types, sexual—preceded by a nuclear fusion—and asexual. The processes leading to sexual reproduction in fungi are still very imperfectly understood. In the first of the main groups outlined below, the Phycomycetes, the process is fairly clear; in the Ascomycetes it is less clear; in the Basidiomycetes nuclear fusions occur which may represent the beginnings of sexual fusion; whilst in the Fungi Imperfecti (by definition) sexual reproduction is unknown. The resulting spore form is known variously as *oospore, zygo-*

spore, ascospore or *basidiospore,* according to its methods of formation.

The asexual spores are usually produced simply by a budding-off process. When such budding takes place from a modified external hyphal branch, the spores are known more correctly as *conidia,* and the branch as a *conidiophore.* Other types of spore exist. *Chlamydospores* are formed by rounding off and thickening of portions of the vegetative hyphæ; *zoospores* are motile asexual spores liberated from an enclosing *sporangium*; and non-motile spores may be produced in a closed *sporangium,* a flask-shaped *pycnidium,* or on a cushion-like *sporodochium.* Several types of spore frequently occur in one and the same fungus.

The variety of shape in spores and spore-bearing structures is limitless, as may be seen on looking through the illustrations in the sections of Rabenhorst's "Kryptogamenflora," relating to fungi. Spores may be single-celled or multicellular, colourless or coloured. The common feature is that the spores are microscopically small, light structures which are readily detached when ripe, and are thus suitable for dissemination by wind, water or animal agency. In size fungal spores vary from spheres 2 μ in diameter to structures 100 μ or more in length (1 μ being one-thousandth of a millimetre). It is quite incorrect to regard spores as the seeds of fungi. They are much less complex structures than seeds, though analogous to the seeds of higher plants in that they can undergo a resting stage and withstand desiccation, factors that enable the fungus to survive exposure to unfavourable conditions.

The majority of fungi are *saprophytes*—that is to say, they are capable of existing on dead organic matter. Many, however, are *parasites* on animals or plants—more especially on plants—and of these parasites some are quite incapable of existing saprophytically. The fungi causing *Rusts* of wheat, for example, have never been cultivated on laboratory culture media. Parasitism by a species of fungus may be possible on a range of host organisms, or may be confined to a single host species; the parasite may pass the whole of its life cycle on one host, or two distinct parts of its life cycle on two different species of host.

An excellent brief description of the principal aspects of the fungi is given by Ramsbottom (1929), and a fuller account of

their structure by Gwynne-Vaughan and Barnes (1937) and Bessey (1935). An advanced treatment of fungal morphology is that of Gäumann and Dodge (1928), whilst for details of individual species it is necessary to go to the compendious works of reference of Rabenhorst (1884-1920), or Saccardo (1882-1931), or to monographs and original papers.

The Main Groups of Fungi.

All classification systems are largely arbitrary, and differences of opinion arise as to the best orderly arrangement of the many thousands of fungal species. The scheme outlined below gives the generally accepted basis for the separation of the larger groups, excluding the Myxomycetes, whose affinity with the fungi is doubtful.

Classification of Fungi.

Vegetative mycelium mostly without cross-walls; spores usually in sporangia		**Phycomycetes.**
Vegetative mycelium divided by cross-walls	Characteristic spores in asci	**Ascomycetes.**
	Characteristic spores on basidia	**Basidiomycetes.**
	Ascospores and basidiospores lacking	**Fungi Imperfecti.**

The **Myxomycetes,** which are not included in the above scheme, are organisms whose vegetative form, known in the aggregate as a *plasmodium*, is simply a mass of protoplasm with no surrounding cell wall. Later in the life history spores are produced. Many myxomycetes occur on old logs, where they become noticeable as slimy masses after wet weather. In general, the group has little economic significance except for *Plasmodiophora*, which is the cause of " Club-root " or " Finger and Toe " in cabbage and turnip; and even this is now considered by many systematists to belong to the lower Phycomycetes.

The **Phycomycetes** may be divided into the *Archimycetes*, in which the mycelium is rudimentary or absent; the *Oomycetes*, in which the mycelium is well developed, motile accessory spores may be found, and sexual reproduction is by means of differentiated organs that give rise to an *oospore* ; and the *Zygo-*

mycetes, in which the mycelium is also well developed, but sexual reproduction is brought about by the union of organs which are not differentiated.

The general name of the group, *Phycomycetes*, is given because the group comprises forms which resemble the green algæ in general structure, although possessing no chlorophyll. The *Archimycetes* include a large number of species parasitic on plants and aquatic animals, but are for the most part of no great importance to man. The chief exception is *Synchytrium*, a species of which causes the dreaded "Wart Disease" of potatoes.

Among the *Oomycetes* are a number of aquatic groups saprophytic or parasitic on plants or aquatic animals; a few species of *Achlya* and *Saprolegnia* are destructive to young fish. The *Peronosporales* are mostly parasites of flowering plants, linked up with aquatic types by the genus *Pythium*, the cause of various "damping off" diseases of seedlings. *Pythium* merges into the genus *Phytophthora*, which is still more terrestrial in habit. Of the many species of this genus causing plant diseases, one of the most harmful is *Phytophthora infestans*, causing "Late Blight" of potato. A closely related type (*Plasmopara viticola*) causes a serious disease of grape vine. These diseases are so widespread that the spraying of potatoes and grape vine with fungicides has become a routine preventive measure. Abundant conidia are produced by such types.

The remaining group, the *Zygomycetes*, includes, in addition to a group of insect parasites, the widely distributed *Mucorales*, practically all of which are saprophytic moulds. *Mucor*, *Rhizopus*, and a few related genera are among the commonest types developing on damp foodstuffs, rotting fruit, or other substrata, and possess characteristic knob-like sporangia. Many possess a marked starch-decomposing ability which has led to the utilisation of such fungi in the fermentation industries. *Thamnidium* develops on meat in cold storage. The phenomenon of *heterothallism* was first observed among the Mucorales; in certain species a mycelium derived from a single spore is one of two types, termed + and − because it is only when hyphæ of opposite sign approach each other that sexual fusion occurs. This is probably equivalent to a sex difference, although without visible differentiation of form. In other groups, however, cases of heterothallism are known

which cannot be easily explained on a sex basis, and " physiological heterothallism," affecting purely vegetative characteristics, is also known.

The **Ascomycetes** are an enormous group characterised by the possession of a special spore form contained in a specialised cell known as an *ascus*. Asci are usually club-shaped cells each containing eight ascospores. The simplest group, and of most interest to the microbiologist, is the *Saccharomycetes*, or Yeasts. Here mycelium is usually absent, the fungus existing in the form of round or oval cells, which multiply in a characteristic manner by budding.

With certain yeasts, under certain conditions of environment, the contents of individual cells may divide up into one to eight endospores; these represent ascospores of the yeasts, and are the ground on which this very characteristic group is placed among the Ascomycetes. Yeasts, owing to their small size and unicellular nature, lend themselves to culture methods applicable to bacteria rather than to the usual mycological technique. Their classification presents great difficulty, even an initial division into yeasts producing ascospores and yeasts not producing ascospores. A recent thorough attempt has been made by Dutch workers (Stelling-Dekker, 1931; Lodder, 1934) to reorganise the classification of yeasts. A rough division for practical purposes is that into " wild yeasts," that show a tendency to form mycelium and have little fermentative powers, and " cultivated yeasts " used in industrial fermentation processes. Of wild yeasts, certain species belonging to the genus *Torula* may cause trouble in the dairy and fermentation industries. Most of the brewing and distilling yeasts are classed under the specific name of *Saccharomyces cerevisiæ* ; the yeast used for baking is usually a distiller's type. For wine-making, strains of *Saccharomyces ellipsoideus* —occurring naturally on the grapes—are the active agents.

In certain other groups of the Ascomycetes the asci are enclosed in spherical tissue-structures called *perithecia*. The common moulds *Aspergillus* and *Penicillium* belong here, though only a minority of species in each of these genera form the " perfect " spore stage. The perithecia-forming Ascomycetes also include the " powdery mildews " (*e.g.*, *Erysiphe*, *Sphærotheca*) which cause a number of plant diseases, the mycelium forming a whitish surface layer on the leaves of the

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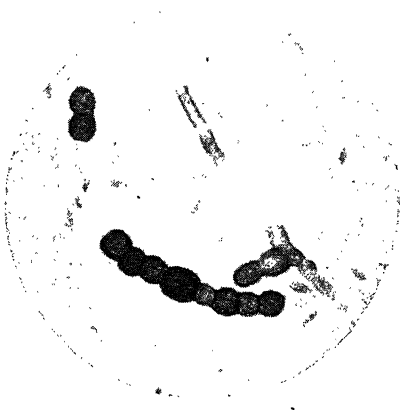

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1. *Aspergillus flavus* SHOWING SPORING HEAD AND MYCELIUM.
2. *Penicillium Roqueforti.*
3. *Oospora* (*Oidium*) *lactis.*
4. *Pullularia* (*Dematium*) *Pullulans.*

(Magnification 400.)

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plant attacked. In contrast to the mildews are the " sooty moulds " (*e.g.*, *Capnodium*), more common on plants in tropical than in temperate countries. The subterranean edible fungi known as truffles may also be mentioned here.

Other groups of the Ascomycetes are included under the name *Discomycetes*, since the asci are borne on a fructification that is disc- or cup-shaped. Among the Discomycetes are included a number of plant parasites (*e.g.*, *Sclerotinia*), but the majority are saprophytes on dead vegetable matter. It is usually the Discomycete type of fungus which is found in the peculiar symbiotic structures known as *Lichens*, familiar as crust-like growths—often brightly coloured—on tree trunks, roofs, and walls (see Smith, 1921). A lichen is conventionally regarded as being composed of a fungus and a green alga living in intimate association (Symbiosis), although the fungus constituent is never found growing independently. Lichens were once of great economic importance as a source of dye-stuffs, of which litmus is still familiar to the laboratory worker. They are also of interest in view of the part they probably play in the gradual disintegration of building stone.

Another section of the Ascomycetes, comprising thousands of species, is the *Pyrenomycetes*; here the structure enclosing the asci is flask-shaped and open at the top. The Pyrenomycetes include a number of plant pathogens (*e.g.*, *Venturia*, *Rosellinia*) of varying importance, certain fungi (*Cordyceps* spp.) parasitic on insects, a few commonly occurring saprophytic " moulds " (*e.g.*, *Chætomium*), and the interesting grain parasite *Claviceps*, the sclerotia or ergots of which are poisonous to animals and yield valuable medicinal principles.

Basidiomycetes.—In this vast group the spores are formed on specialised cells known as *basidia*; these are club-shaped cells, each bearing two or four spores supported on short stalks.

Among the more microscopic forms are organisms causing the very important crop diseases commonly known as Smuts (*e.g.*, *Ustilago*, *Tilletia*), and Rusts (*e.g.*, *Puccinia*), whose ravages cause a steady drain on the yield of cereal crops all over the world. Such species do not grow at all, or give only a much reduced growth, except on the living tissues of the host plants.

The " higher " Basidiomycetes, on the other hand, include a

large number of saprophytic species, and assume a great variety of form, the larger types being popularly known by such names as mushrooms, toadstools and bracket fungi. Some crop disease forms, notably *Corticium salmonicolor*, causing "Pink Disease" of rubber and tea crops, and *Stereum purpureum*, causing "Silver-leaf" diseases of fruit trees, also occur among the higher Basidiomycetes. The gill-bearing fungi (*Agaricaceæ*) are represented by the familiar field mushroom *Psalliota campestris*. Examination of the radiating gills under a high magnification shows that they serve to provide a large surface on which the basidiospores are produced in vast numbers. *Amanita phalloides*, not dissimilar to the untrained eye, contains a most powerful poison, and is responsible for the majority of deaths due to eating fungi.

Most of the serious timber disease fungi, both those on felled timber and on the living tree, belong to another sub-group, the *Polyporaceæ*. Here the large spore-bearing surface is provided, not by radiating plates, but by numerous pores. Such bracket fungi include the genera *Fomes*, *Polyporus* and *Trametes*, which are parasitic on living trees.

Merulius lachrymans causes "dry rot" of structural timber, finally resulting in complete crumbling of the wood. Similar but less serious rots are caused by several related species.

The common puff-ball fungi represent another sub-group, the *Gasteromycetes*, all of which are saprophytic.

The **Fungi Imperfecti,** as already stated, are a make-shift group in which conidia are the only form of spore known. From time to time, however, the "perfect" spore stage of such a species is found, and the species is then transferred to its proper position, which is almost invariably one of the sub-groups of the Ascomycetes. This somewhat untidy state of things is very repugnant to the academic mind, and most books on systematic mycology dismiss the vast group of Fungi Imperfecti in one or two pages, perhaps lest they prove unsettling to students. The fact remains, however, that these types constitute the majority of those encountered by the economic microbiologist, and some means of identification is essential. Bisby and his collaborators (1933), in their investigation of soil fungi, found that over 90 per cent. of the isolations were imperfect fungi; when moulds of butter were under consideration (1933*a*), the percentage rose to 99 per cent.

Classification of this group is necessarily artificial, but the three main sub-groups generally recognised are the *Sphæropsidales*, the *Melanconiales* and the *Hyphomycetales*.

The *Sphæropsidales* include species in which the spores are borne within flask-shaped *pycnidia*. A large number of plant parasites belong to this sub-group, and from time to time certain species are found to produce perithecia and are transferred to the *Pyrenomycetes*.

In the *Melanconiales* the conidiophores are formed in a closely aggregated sub-epidermal layer known as the *acervulus*. Most of the species are parasitic or saprophytic on plant tissues.

The *Hyphomycetales* possess conidia borne on freely developing conidiophores which develop outside the host material. This sub-group includes in addition to parasitic forms a large number of saprophytic species of economic interest. Many possess a dark pigmented mycelium and are particularly troublesome in causing stains on foodstuffs or fabrics.

Important genera of the Fungi Imperfecti are *Cladosporium*, *Alternaria*, *Fusarium*, *Oospora*, *Phoma*, *Helminthosporium* and *Colletotrichum*.

Among the imperfect fungi, but not in the three main groups given above, one must also place two vague groups of fungi: (1) The "mycelia sterilia," in which no spores are found. The best known type of this group is *Rhizoctonia*, a form-genus with characteristic mycelium, several species of which occur in soil and are capable of causing plant diseases. (2) The small group of fungi causing dermatomycoses, or skin diseases, in man and animals.

The Identification of Fungi.

To give some idea of the difficulty involved in identifying a fungus with accuracy, the following very moderate estimate of the number of well-described species is quoted from Gwynne-Vaughan and Barnes (1937):

Phycomycetes	1,000
Ascomycetes	over 16,000
Basidiomycetes	over 14,000

No estimate is given for the Fungi Imperfecti. These figures may be compared with the larger estimate of Bessey (1935), who gives:

Ascomycetes	35,000
Basidiomycetes	24,500
Fungi Imperfecti	25,000

It follows that exact determination is a matter for an expert, and, moreover, an expert in a particular group of fungi. Otherwise it is better to err on the side of caution; a wrong identification is worse than useless, since it is actually misleading. A careful microscopic examination and reference to suitable literature will, however, usually give an idea of the approximate position of the organism under investigation.

Pure cultures of most of the fungi capable of growing on laboratory media can be obtained at a very small charge from the National Collection of Type Cultures, Lister Institute, London, or from the Centraalbureau voor Schimmelcultures, Baarn, Holland. These institutions are also in a position to suggest a suitable specialist for advice on any particular group.

In the case of plant parasites, once the fungus has been allotted to its approximate position, it is customary to shorten one's labour by reference to a host index to see what parasites on that host have been previously recorded. Identification of fungi on other special substrata may sometimes be facilitated by similar short cuts.

Clements and Shear (1931) give an up-to-date summary of generic characters. The volumes of Rabenhorst (1884-1920) or Saccardo (1882-1931) may be consulted for fuller description of the species, or Sorauer's "Handbuch" for plant parasitic forms. Monographs are available for different groups of genera —*e.g.*, Thom (1926, 1930), Lendner (1908)—and frequently recourse must be made to original papers. A good account of the mould fungi is given by Smith (1938). Bessey (1935) gives a recent summary of useful references, and the monthly *Review of Applied Mycology* published at Kew forms an invaluable guide to recent literature. For an account of the economic uses of fungi see Ramsbottom (1936). Other helpful sources of information are Butler (1918), Fitzpatrick (1930), Henrici (1930), and Lindau (1922).

REFERENCES

BESSEY, E. A. (1935): Textbook of Mycology. Philadelphia.
BISBY, G. R., JAMES, N., AND TIMONIN, M. (1933): Can. J. Res., 8, 253.
—— JAMIESON, M. C., AND TIMONIN, M. (1933*a*): Can. J. Res., 9, 97.

BUTLER, E. J. (1918): Fungi and Disease in Plants. Calcutta.
CLEMENTS, F. E., AND SHEAR, C. L. (1931): The Genera of Fungi. New York.
FITZPATRICK, H. M. (1930): The Lower Fungi: Phycomycetes. New York.
GÄUMANN, E. A., AND DODGE, C. W. (1928): Comparative Morphology of Fungi. New York.
GWYNNE-VAUGHAN, H. (1922): Fungi: Ascomycetes, Ustilaginales, and Uredinales. Cambridge.
——, AND BARNES, B. (1937): The Structure and Development of the Fungi. Cambridge.
HENRICI, A. T. (1930): Molds, Yeasts, and Actinomycetes. New York.
LENDNER, A. (1908): Mucorinées de la Suisse. Berne.
LINDAU, G. (1922): Die Mikroskopischen Pilze. Berlin.
LODDER, J. (1934): Monographie der Hefearten. II. Die Asporogenen Hefen. Amsterdam.
RABENHORST, L. (1884-1920): Kryptogamenflora, vols. 1-10. Leipzig.
RAMSBOTTOM, J. (1929): Fungi. London (Benn's Sixpenny Series).
—— (1936): Chem. and Ind., 55, 717.
SACCARDO, P. A. (1882-1931): Sylloge Fungorum, vols. 1-25. Pavia.
SORAUER, P. (1928, 1932): Handbuch der Pflanzenkrankheiten, vols. 2 and 3. Berlin.
SMITH, A. L. (1921): Lichens. Cambridge.
SMITH, G. (1938): Introduction to Industrial Mycology. London.
STELLING-DEKKER, N. M. (1931): Monographie der Hefearten. I. Die Sporogenen Hefen. Amsterdam.
THOM, C. (1930): The Penicillia. Baltimore.
—— AND CHURCH, M. B. (1926): The Aspergilli. Baltimore.

CHAPTER III

THE BACTERIA

THE bacteria form a large group of unicellular forms, which in their manner of life bear a general resemblance to the fungi. They are usually clearly distinguished from the fungi, but, as throughout the whole biological system, borderline forms exist. The *Actinomycetes*, for instance, may for ordinary purposes be regarded as intermediate between fungi and bacteria. Actinomycetes form a branched mycelium resembling that of the fungi, but of finer structure, the thickness of the threads of hyphæ being more comparable with that of many rod-shaped bacteria. Reproduction is by means of conidia and by breaking up of the hyphæ into short lengths. Such forms, when liberated, are often indistinguishable from bacteria.

Certain other thread-like types of bacteria—*e.g.*, the "Iron Bacteria"—are only with difficulty distinguished from fungi or blue-green algæ, while causal organisms of diphtheria and tuberculosis, which were formerly regarded as typical bacteria subject to "involution forms"—*i.e.*, showing deviation from the usual rod shape—are now classed by many workers among the Actinomycetales. Certain of the Fungi Imperfecti—*e.g.*, species of *Cytospora*—have minute spores which may be easily mistaken for bacteria.

Our knowledge of bacteria is still very incomplete. Bergey (1939) describes 1,335 species, of which about 10 per cent. are pathogenic to man, animals or plants. The comparatively high proportion of pathogenic species merely indicates that attention has been specially devoted to these. The industrial worker who tries to identify a random collection of non-pathogenic isolations soon realises that many of them have not hitherto been described, and can be allotted only an approximate systematic position. Many bacteria are of use to man in breaking down animal and vegetable debris and in promoting soil fertility, and an ever-increasing number are

finding applications to technical processes. Some are a constant source of damage to natural and artificial products, and the remainder—so far as our present imperfect knowledge goes —do not affect man's activities to any very great extent.

It is difficult to realise the extreme smallness of these organisms. Two illustrations may help to give some indication of their size and numbers. Many bacteria are roughly cigar-shaped. Suppose that such a bacterium of average size were magnified to the size of a real cigar and that a man were magnified on the same scale. The smoker would then be twenty miles tall. Again, a liquid frequently contains several million bacteria per ml. If we assume that the bacteria are spheres 1 micron or μ (*i.e.*, $\frac{1}{1000}$ mm.) in diameter—and this represents a rather large type—1 ml. composed entirely of them would contain a million million organisms. Thus, if a sample of milk contains one million bacteria per ml., only one-millionth of the volume of the milk is occupied by micro-organisms.

Bacteria may be roughly considered as spherical, rod-shaped or screw-shaped. The rod forms are long or short, some being so short that they cannot readily be distinguished from the spherical cocci. The screw-shaped organisms range from "comma" forms to long spirals and are the least frequent. In size the spherical forms average about 1 micron in diameter; the rods range from 0·2 to 2·0 μ in thickness and 1 to 5 μ or even more in length, according to the species, and may adhere together in short or long chains. In addition to the above types there are the more complex thread-like forms, each consisting of a chain of rods end to end, within a common gelatinous envelope often many hundreds of microns in length. In comparison with the bacteria, fungal spores and yeast cells are usually five to ten times larger in diameter.

Owing to the extremely small size of bacteria little structural detail is visible, even under the highest power of the microscope. The organism consists of the living substance protoplasm bounded by a membrane of protein nature, probably a specialised form of the protoplasm itself. Granules staining with methylene blue are not infrequent, and certain denser structures taking nuclear stains have been described for certain species (Stoughton, 1929). The latter, however, show none of the complex structure of the nuclei of fungi and higher

organisms. Some species develop a mucilaginous envelope called a *capsule*. This often causes the individual bacteria to adhere together, forming a slimy mass (*zooglœa*) which may give rise to an increase in viscosity of the fluid medium in which the organisms are growing. The production of capsules is frequently controlled by the nature or reaction of the medium. Certain economic consequences of capsule formation—ropy milk and the clogging of pipes in sugar factories or paper mills—will be referred to in later chapters. Sometimes, as in vinegar manufacture or sewage disposal, capsule formation may be profitably utilised.

Movement.—Many bacteria are capable of locomotion in a fluid medium. Whip-like flagella are formed, and though these themselves are beyond the limits of vision, they may be demonstrated by special staining techniques involving the precipitation of stain on them. It is a strange fact that, however crowded the bacteria may be, their flagella do not seem to become entangled. Bacteria are so small that a trembling motion is imparted to them by constant bombardment of molecules in a liquid medium. Motility due to flagella is continuous and in all directions, and may readily be distinguished from this "Brownian movement" and from any streaming movement of the liquid under examination. Since the position of the flagella is often used by some systematists as a diagnostic character, it is necessary to distinguish organisms with a single polar flagellum (monotrichous), with a flagellum at each end (amphitrichous), with a polar tuft (lophotrichous), and with flagella scattered all over the cell (peritrichous).

Reproduction.—Bacteria reproduce by a process of simple cell division. Nothing corresponding to sexual reproduction is definitely established. It has been claimed, however, that their life cycle is quite complex; stages that have been reported include extremely minute and in some cases filtrable bodies, the union of two or more cells, and the fusion of protoplasm from many cells later resolving into regenerative units and ultimately into normal bacteria. Upon reaching adult size the protoplasm retracts and a septum is formed across the cell (transversely in the case of rod-shaped organisms) which divides it into two approximately equal daughter cells each capable of existing as an individual. These grow to full size

and the dividing process is repeated. In some species the many individuals so produced remain closely attached to one another, thus giving rise to clumps or chains. In the spherical forms (*cocci*) the cells after division may again divide in the same plane, leading to short or long chains of *streptococci*; or the second division may be in a different plane from the first, leading to flat plates of four (*tetracocci*) or to less symmetrical clusters (*staphylococci*). In the *sarcina* type the third division is at right angles in another dimension, so that small packets or bales are formed.

So long as conditions are favourable the organisms multiply at a very rapid rate, dividing perhaps every twenty minutes. After a time, however, the process is checked, either by exhaustion of the food supply or by the production of by-products that inhibit growth. The latter process is known as *staling*. Eventually death occurs, and the bacteria become decomposed by their own enzymic influences (autolysis), unless such action is checked by heat, desiccation, or chemical agency.

Resistance to Unfavourable Conditions.—Bacteria show great variation in their capacity to withstand unfavourable conditions. Certain types are capable of forming *spores*, which, like those of the fungi, form a simple resting stage, but are much more resistant than fungal spores to heat, desiccation and chemical influences. Bacterial spores are refractile bodies enclosed in a tough membrane; only one spore is formed by each cell, and this is characteristic in size and position.

The vegetative forms, although less resistant than spores, are nevertheless in many cases able to survive unfavourable conditions, especially when surrounded by protective colloidal material, as is the case with soil organisms.

Oxygen and Temperature Requirements.—Many bacteria resemble the fungi in requiring an ample supply of oxygen for growth. Such forms are termed *aerobic*, but there are also *anaerobic* forms which grow best in the absence of air, obtaining their energy by intramolecular decomposition of carbohydrates or other organic compounds. Between these types are the facultative anaerobes, which can exist either in the presence or absence of air.

The physiological and biochemical processes of bacteria are of course greatly accelerated by rise of temperature, but for each organism there is an optimum temperature above which

growth falls off rapidly and soon ceases. For most bacteria this optimum temperature is between 20° and 40° C., and it is usual to maintain incubators at 20° for " cool " organisms and 37° for " blood-heat " organisms.

The lower limit of growth is at about 5° to 10° C. for most organisms, hence the value of cold storage for food preservation. Certain types still multiply at low temperatures, but slowly.

The maximum temperature is usually only a few degrees above the optimum. The extraordinary group known as the *thermophilic* bacteria are capable of growing at 65° C., or in some cases even at 75° C.

Variation.—Many species of bacteria often show morphological or physiological variations. A particularly common one is the formation of smooth (S) and rough (R) surface colonies on solid media, which may sometimes be correlated with other variations—*e.g.*, in pathogenicity—of importance to medical workers.

For fuller information regarding form and function of bacteria, see Henrici (1934) and the series of volumes published by the Medical Research Council (1931).

Classification.

The classification of bacteria has not been so completely worked out as that of the fungi. This is partly because their study is of much more recent date, and partly because of the difficulties inseparable from their small size. Bacteria are so minute that they present few details of structure capable of being used for classification purposes, and one is compelled to fall back on the less constant criteria of biochemical characteristics. Thus in addition to appearance on culture media, size, shape, motility, and spore formation, diagnostic features include the production of acid or gas from various sugars, gelatin liquefaction, indole formation from proteins, reduction of nitrates to nitrites, and similar reactions.

The scheme put forward in Bergey's manual (the first edition of which appeared in 1923) is that published by a Committee of the Society of American Bacteriologists. It does not meet with the unqualified approval of European workers, many of whom cling to the older system of Lehmann and Neumann, where, for example, all sporing rods are called *Bacillus*, and all

non-sporing rods *Bacterium*. Bergey's system also includes all spore-formers in the *Bacillaceæ*, but the lactic organisms, though rightly included under the non-sporing *Bacteriaceæ*, are for some reason given the generic name of *Lactobacillus*. Many of the American names for genera are cumbersome and unpleasant to the ear: the *Bacterium coli* of European workers becomes *Escherichia coli*. A further criticism of Bergey's classification is that one of the criteria by which certain genera are distinguished is the presence and nature of the flagella. As these are seen only after special staining methods possible to the expert alone—even then frequently of doubtful interpretation—they form an unsatisfactory criterion for general use.

Nevertheless, Bergey's manual forms the most comprehensive handbook available for identification purposes, and his system is adopted in the brief account which follows.

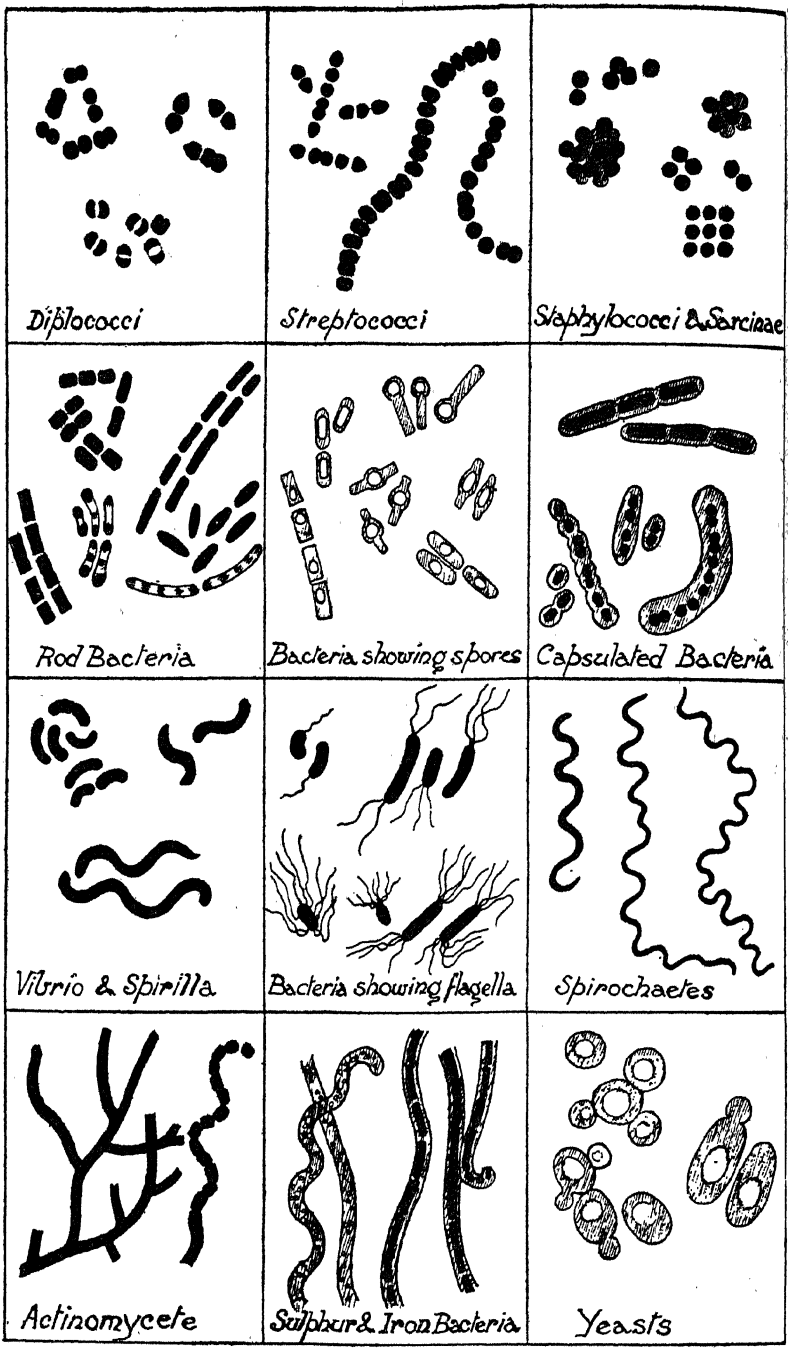
Quite recently an entirely new scheme for the "rational" classification of bacteria has been put forward by Kluyver and Van Niel (1936), and this appears to present certain advantages over the older systems.

Brief Summary of the Groups of Bacteria.—For the sake of simplicity the arrangement in the fourth edition (1934) of Bergey's manual has been retained. The 1939 edition introduces further modifications, especially in the *Eubacteriales*, which are divided into twelve families instead of the five in the scheme below. Another change is that the genera Streptococcus and Diplococcus are transferred to the *Bacteriaceæ*, where they form a sub-group with *Lactobacillus*.

The bacteria may be divided into six groups or orders, of which the order *Eubacteriales*, the "true" bacteria, is by far the largest and most important.

Order 1. Eubacteriales.—This consists of simple, non-branching forms which are spherical, curved or straight rods. The organisms are not aggregated into sheathed filaments and do not contain granules of iron or sulphur or bacteriopurpurin, a purple pigment found in members of the fourth group. The group contains five families as follows:

(*a*) *Nitrobacteriaceæ*.—Organisms in this family are rod-shaped but non-sporing. They may have polar flagella. Energy for growth is often obtained from simple compounds. Some members—viz., species of the genera *Nitrosomonas*,



BACTERIA AND YEASTS.

Nitrobacter and *Azotobacter*—play an important part in soil fertility, while species of *Rhizobium* live symbiotically in the roots of leguminous plants, where they fix free nitrogen, and are thus also of use to the farmer. Species of *Acetobacter* oxidise alcohol to acetic acid and are used in vinegar manufacture.

(*b*) *Coccaceæ*.—These forms are spherical, non-motile, non-sporing, usually Gram-positive and frequently pigmented. As with all pigmented bacteria, except those mentioned in Group 4, the colour is only shown when the cells are massed, as in colony formation on solid nutrient media. The cells may be aggregated into pairs (*Diplococcus*), chains (*Streptococcus*), irregular masses (*Staphylococcus*, *Micrococcus*), or regular packets of eight or more (*Sarcina*). Species of economic importance are *Streptococcus lactis*, the organism commonly causing souring of milk; and *Str. cremoris*, which is used as a " starter " in the preparation of dairy products. Pathogenic types include *Staphylococcus aureus*, associated with septicæmic conditions; *Str. mastitidis*, which is the chief cause of mastitis (udder inflammation) in cows; and *Diplococcus pneumoniæ*, the commonest cause of lobar pneumonia.

(*c*) *Spirillaceæ*.—These are rigid, non-sporing, spiral cells, motile with polar flagella. They are common in water and putrid infusions. Some are intestinal, and some species of the genus *Vibrio*—*e.g.*, *V. comma*, the cause of cholera—are pathogenic. The family contains no member of particular importance to industry.

According to Bunker (1936), anaerobic or microaerobic species of *Vibrio*—*e.g.*, *V. desulphuricans*—are responsible for the widespread reduction of sulphates in nature. These are common in sea, river muds and soil, and may act deleteriously in producing hydrogen sulphide in water of coal-gas holders, in filter beds, and in wood pulp and paper manufacture. Corrosion of stone, concrete and metal may also be due to them. Under anaerobic conditions they produce black colonies in the synthetic medium described in Chapter VI.

(*d*) *Bacteriaceæ*.—This family contains the majority of genera and species, and is probably the most important of all. The organisms are non-sporing rods, usually Gram-negative and motile. They have a complex metabolism, requiring proteins as a source of food. Amongst the most interesting genera are the following:

(i.) *Escherichia* and (ii.) *Aerobacter*.—These organisms are short, Gram-negative rods, capable of fermenting lactose with the production of acid and gas. They are facultative anaerobes. The species as a whole constitute the commonly designated *coli-aerogenes* or coliform group. They are distinguished by their biochemical reactions. *Escherichia* (*Bact.*) *coli* is motile and mostly intestinal, while *Aerobacter* (*Bact.*) *aerogenes* is essentially a saprophyte, its normal habitat being the soil and dead vegetable matter. It is, however, found in fæcal matter. The presence of these organisms in fæces, coupled with their capability of growing in a medium containing bile salt, with the production of acid and gas, constitutes a useful basis for the detection of fæcal contamination in water, dairy and other food products. For convenience the two main species will be referred to as *Bact. coli* and *Bact. aerogenes* in subsequent chapters.

(iii.) *Salmonella*.—Many species of this genus occur in the intestinal canal of animals and in acute inflammatory conditions. Certain species and their relation to food poisoning are discussed in Chapter XII.

(iv.) *Eberthella*.—*E. typhosa* (*Bacterium typhosum*) is the cause of typhoid fever. Nearly all the species are inhabitants of the intestinal canal of animals.

(v.) *Shigella*.—Species occur in the intestinal canal and in dysenteric conditions. Many are pathogenic to man and animals.

(vi.) *Proteus*.—Many of these organisms play a prominent part in the decomposition of proteins.

(vii.) *Brucella*.—The three species of this genus are pathogenic. *B. abortus*, *B. melitensis* and *B. suis* cause abortion in cattle, goats and swine respectively. All can cause undulant fever (brucellosis) in man.

All the foregoing are Gram-negative rods.

(viii.) *Lactobacillus*.—These are either aerobic or anaerobic, Gram-positive, non-sporing, non-motile rods capable of producing lactic acid from lactose. They are invariably found in dairy products and other lactose-containing foods. Two groups of organisms

are distinguished: those producing chiefly lactic acid and those producing by-products in addition —*e.g.*, carbon dioxide, alcohol, acetic acid.

Species of the genera (ix.) *Erwinia* and (x.) *Phytomonas* are pathogenic to plants.

(*e*) *Bacillaceæ.*—These are motile or non-motile, sporing, usually Gram-positive rods having peritrichous flagella. They have a complex metabolism, and many are able to decompose proteins with evolution of simple products like ammonia. Being spore-forming organisms, their control presents considerable difficulty. The family is divided into two genera, which differ in their oxygen requirements: (i.) *Bacillus* (aerobic) and (ii.) *Clostridium* (anaerobic).

Soil-drainage water organisms of the *Bacillus subtilis-mesentericus* group are well known by virtue of their proteolytic activities. Many are responsible for the disintegration of animal textile hairs—*e.g.*, wool—and play a conspicuous part in tanning operations. Varieties of *Bac. mesentericus* in flour have been found responsible for "ropiness" in bread. *Bac. anthracis*, the cause of anthrax, produces a powerful toxin.

Many species of *Clostridium* are pathogenic—*e.g.*, *Cl. tetani* (lockjaw), *Cl. chauvei* (blackquarter), and *Cl. œdematis-maligni* (malignant œdema). *Cl. welchii* and *Cl. sporogenes* are intestinal forms, and their detection in potable waters is considered to indicate fæcal contamination. They are the chief organisms associated with gas gangrene. By penetrating through the bowel wall at the death of an animal they become largely instrumental in bringing about the putrefaction of carcasses. *Cl. botulinum*, a saprophyte on organic matter—*e.g.*, prepared meat foods—produces a powerful endotoxin which gives rise to the usually fatal disease of botulism.

Order 2. Actinomycetales.—This group contains the families *Actinomycetaceæ* and *Mycobacteriaceæ*. The cells are elongated and frequently filamentous (thread-like), with a tendency towards the formation of branches. They do not

contain iron, sulphur or purple pigment. According to McCarter and Hastings (1934-35) it would appear that at least some members of the genus *Mycobacterium* do not show true branching. Perhaps the older classification is more correct, and this genus, and *Corynebacterium*, are more correctly placed in the family *Bacteriaceæ* in Order 1.

(i.) *Actinomycetaceæ.*—The principal genus is *Actinomyces*, and, as already mentioned, this is classed by many as being separate from the Bacteria. The branched threads usually give rise to a mycelium, the colonies in both liquid and solid media being mould-like. Conidia are produced by some species, often on spiral, aerial branches. The threads are non-motile and usually Gram-positive. The Actinomycetes usually require complex proteins as food and grow only slowly on culture media. Saprophytic Actinomycetes are abundant in the soil and are mostly aerobic. Their cultures emit a pronounced earthy smell, and it is possible that the odour of freshly turned soil is due to them. A few species are animal parasites, and of these some are anaerobic. *Actinomyces bovis* is the cause of actinomycosis (lumpy jaw) in cattle, and a variety of this is responsible for actinomycosis in man. Macroscopic "colonies" or growths may readily be detected in the resulting pus. The periphery of such growths is occupied by very enlarged club-shaped cells readily seen in cross-sections of affected material and pus smears. *Act. scabies* is the causal organism of common scab in potatoes. *Act. necrophorus* is the necrosis bacillus causing necrosis or degeneration of soft tissues in cattle. *Erysipelothrix rhusiopathiæ* is the cause of swine erysipelas. *Act. madurœ* appears to be the causal organism in certain forms of "Madura Foot," a serious disease which occurs principally in tropical countries, and for which no effective treatment has been discovered.

(ii.) *Mycobacteriaceæ.*—This group consists largely of parasitic forms, the chief genera being *Mycobacterium* and *Corynebacterium*. The characteristic

of members of the genus *Mycobacterium* is that the organisms are "acid fast"—they retain carbol-fuchsin stain even in the presence of 25 per cent. sulphuric acid. They are mostly slender, Gram-positive, non-motile rods. Adult cells may stain irregularly, and frequently when so treated have a beaded appearance. Their growth in culture media is slow.

Many of these organisms are parasitic; the most important is *Mycobacterium* (*Bacterium*) *tuberculosis*, varieties of which give rise to tuberculosis in man, cattle, swine and poultry. Other harmful species are *M. paratuberculosis*, which causes Johne's disease in cattle, and *M. leprœ*, the cause of leprosy. Some members are non-pathogenic, and their presence in milk, for example, may lead to erroneous conclusions. The genus *Corynebacterium* consists of aerobic, non-sporing, Gram-positive, slender rods, either curved, club-shaped, or straight, and of variable length. When stained, as, for example, with methylene blue, more deeply coloured granules are conspicuous. *Corynebacterium diphtheriæ* is the causal organism of diphtheria. *Actinobacillus* (*Pfeifferella*) *mallei* is the cause of glanders, and infects horses, man and other animals. *Act. lignieresi* causes actinobacillosis in cattle, a disease which resembles actinomycosis, but differs from it in that the bone tissue is not affected and in being less responsive to chemical treatment. Species of *Cytophaga* are noteworthy in their ability to decompose cellulose.

ORDER 3. CHLAMYDOBACTERIALES.—Organisms in this group are algæ-like and typically water forms. The cells are arranged end to end in filaments which are frequently surrounded by a membranous sheath in which granules of iron oxides may be embedded. Conidia are produced in some species from the tips of the filaments. No sulphur granules or purple pigment are present. Many species grow in stagnant water, and others in running water which contains soluble organic matter and iron salts. Here they give rise to thick masses, brownish in colour owing to the deposits of iron oxides. Col-

lectively they are called the "*Iron Bacteria*," though not all precipitate iron. Soluble iron bicarbonate is absorbed in the bacterial cells, and this is oxidised to ferric hydroxide, which is stored in the sheath. Bog-iron ore and ferruginous ochre owe their presence to these bacteria. The organisms frequently cause trouble by clogging water pipes, and methods for their control in relation to water supplies will be dealt with in Chapter XII. Well-known genera are *Crenothrix* and *Sphærotilus*.

ORDER 4. THIOBACTERIALES.—These are the so-called "Sulphur Bacteria." Many are elongated filaments, often with a well-defined sheath. Conidia are frequently found, and free sulphur, iron or purple pigment is often present. The organisms grow in water containing sulphuretted hydrogen, which they oxidise to sulphur. If the supply of the former is cut off the deposited sulphur is further oxidised, both these processes supplying energy. Two main groups are distinguished: (*a*) Colourless filamentous types—*e.g.*, *Beggiatoa* and *Thiothrix*; and (*b*) "Purple Bacteria," having bacteriopurpurin.

Beggiatoa species live in stagnant water. The filaments are sheathless and exhibit motility, which is due to a wriggling movement. The organisms are to be seen in sewage tanks.

Thiothrix species live in running water, the sheathed filaments being attached to submerged objects.

The *Purple Bacteria* can withstand greater concentrations of hydrogen sulphide and of organic matter than the filamentous forms. Their purple pigment enables them to assimilate atmospheric carbon dioxide, the stimulus probably being supplied by ultra-red radiation. These organisms are also inhabitants of stagnant water.

ORDER 5. MYXOBACTERIALES.—This is a curious and isolated group of organisms living on dung and rotten wood. They are of little economic significance, so far as is known.

ORDER 6. SPIROCHÆTALES.—These organisms resemble protozoa in many characters. The cells are usually slender, flexuous spirals. In most species multiplication takes place by a longitudinal division. Some spirochætes are common in foul waters and sewage. Many are parasitic—*e.g.*, *Treponema pallidum* causes syphilis, while species of *Borrelia* cause various relapsing fevers, being transmitted by bugs and ticks.

Viruses.

A virus is an infective agent below the limit of microscopic visibility. Virus diseases of animals and plants will be referred to again in Chapters XII. and XIII.

Viruses are usually capable of passing through porcelain or similar filters, which retain even the smallest bacteria. Multiplication of viruses only takes place in the presence of suitable living tissue. Like enzymes, they are somewhat more resistant than bacteria to the action of physical and chemical agents.

The nature of viruses is still in doubt. One view is that they are simply bacteria of ultra-microscopic size. See Smith (1937), Fairbrother (1934), Gardner (1931).

Bacteriophage.—Various species of bacteria have been observed to be susceptible to dissolution by specific invisible agents. Clear zones may develop in bacterial colonies, or clearing may occur in broth cultures. The infective agent is capable of reproduction in the presence of the appropriate bacterium. There seems little reason for regarding a bacteriophage as anything other than a special form of virus which is capable of causing the total destruction of the host.

An alternative view is that the phage is developed by the bacteria themselves—*i.e.*, a kind of autolysing enzyme stimulating the production of further enzyme.

REFERENCES

Bergey, D. H. (1939): Determinative Bacteriology. Baltimore.

Bunker, H. J. (1936): D.S.I.R. Chemistry Research, Special Report No. 3. H.M. Stationery Office.

Fairbrother, R. W. (1934): Filterable Viruses. London.

Gardner, A. D. (1931): Microbes and Ultramicrobes. London.

Henrici, A. T. (1934): The Biology of Bacteria. London.

Kluyver, A. J., and van Niel, C. B. (1936): Zentr. f. Bakt. II. Abt., **94**, 369.

Lehmann. K. B., and Neumann, R. O. (1927): Bakteriologische Diagnostik. Munich.

McCarter, J. R., and Hastings, E. G. (1934): J. Bact., **27**, 41.

—— (1935): *Ibid.*, **27**, 503.

Medical Research Council (1931): System of Bacteriology. 9 vols. H.M. Stationery Office.

Smith, K. M. (1937): Recent Advances in the Study of Plant Viruses. London.

Stoughton, R. H. (1929): Proc. Roy. Soc., B., **105**, 469.

Tanner, F. W. (1938): Bacteriology. New York.

CHAPTER IV

APPARATUS AND STERILISATION

LABORATORY CONDITIONS.—The desirability of a clean, still atmosphere has already been stressed. A north lighting is desirable, although as regards microscope work there is much to be said for the uniform conditions of an artificial source of illumination. Working benches for biological laboratories are usually too high, being based on the requirements of chemical workers, who stand more often than they sit. For microscope work a bench of 2 feet 6 inches to 2 feet 8 inches in height is more comfortable, and another bench or wall table 3 feet high may be provided for standing work. There should be ample knee clearance below the working bench, which should for preference be supported from the wall and not from the floor. Cupboards beneath the bench are troublesome and the contents are usually forgotten; apparatus is better kept on racks or shelves and in glass-fronted cupboards. A washing sink should be provided with draining boards and pegs for drying glassware. A shelf for stains may conveniently be placed above the draining board.

Sterile Chamber.—Some laboratories are equipped with a special "sterile room," fitted with a double door, and a ventilation hole with a cotton-wool filter. Walls, floor, and working bench are of smooth washable material, and are wiped down periodically with dilute "lysol" solution, so that the atmosphere is dust-free as well as draught-free.

The same principle may be applied on a smaller scale by using a glass "sterile hood" constructed like a large balance case with a sliding glass front. The inside is swabbed with 50 per cent. alcohol an hour or two before use, and during use the front is raised just sufficiently to allow the operator's hands to enter the hood.

Microscope and Accessories.—A good quality microscope with substage condenser, 5x and 10x eyepieces, and 16, 4, and 2 mm. objectives fitted in a triple nosepiece, is sufficient for all ordinary purposes. A 20x eyepiece and an 8 mm. objective

may be added to the above, and a mechanical stage is highly desirable. An eyepiece micrometer should be kept with the microscope, or left permanently in one of the eyepieces; by means of a stage micrometer it may be calibrated so that for a definite tube length and objective the number of microns ($\frac{1}{1000}$ mm.) corresponding to one division in the eyepiece is known. A card giving this figure for each of the objectives in use should be kept at hand, so that the size of any object under examination can be ascertained without delay.

Apochromatic objectives, used with "compensating" oculars, give the best definition, but are not essential for routine work. With the 2 mm. ($\frac{1}{12}$ inch) objective, cedar-wood oil must be placed between the lens and the slide under examination. Lenses should be wiped when necessary with a rag of silk or soft cotton, or with the lens paper sold for this purpose; a trace of xylol or benzol may be used to remove any immersion oil that has dried on the lens. Alcohol should not be applied to any part of the microscope.

As a source of artificial lighting a 60-watt opal bulb enclosed in a suitable housing is very satisfactory, and when a more intense illumination is required the Pointolite lamp gives good results.

Microphotography. — In taking microphotographs (the American introduction "photomicrographs" is a more cumbersome and equally incorrect designation) it is often desirable—in order to secure maximum depth of focus—to use the lowest magnification necessary to bring out the desired detail, and to enlarge the photograph later if required. The magnification should be obtained as far as possible through the objective and not through the eyepiece. At very high magnifications the slightest jolt in exposing the plate is apt to throw the microscope out of focus. Small cameras of the Leitz "Makam" type, which fit into the draw tube of the microscope, obviate this difficulty, for by means of a movable prism the focussing can be checked through a side tube both before and after exposure, whilst the dark slide is still open.

Space does not permit further notes on microphotography, but it is worth calling attention to the advantages of a focussing screen of ground glass for securing even illumination, and a screen of clear glass for accurate focussing by means of a lens mounted on a stand and focussed on the lower surface of the

glass screen. Some workers use a ground-glass screen with a small central disc of clear glass, or with a cover-glass fixed to the screen by means of Canada balsam, this giving the same effect.

Glassware.—In addition to the usual apparatus of a chemical laboratory, essentials are an ample supply of test tubes—the thick glass, lipless variety for preference; flasks (conical) and beakers of various sizes; an ample supply of graduated 10 ml. and 1 ml. pipettes, both " blow out " and with lower graduation marks, and of petri dishes.

A supply of sterile pipettes should be kept at hand, and these are conveniently prepared as follows: Insert loosely a small cotton plug for about ½ inch into the open end of the pipette, and cut or flame it off flush with the end. Then take a strip of paper (thin grease-proof paper as sold for shelving is suitable) about 2 inches wide, and after doubling it over the outlet end of the pipette, wind it spirally until the pipette is covered to the plugged end, when the paper may be secured with a twist. Such pipettes are sterilised in the dry oven and then stored in a canister or drawer until required, when the paper (made somewhat brittle by the heat of sterilisation) is easily broken without the operator touching the lower end of the pipette.

Petri dishes are flat circular glass dishes covered by similar dishes of a slightly larger diameter. The lid is loosely fitting, but in a still atmosphere is sufficient to prevent the entrance of air-borne infecting organisms. A supply of sterile petri dishes of a suitable diameter, say 4 inches, and of sterile pipettes should be maintained. The dishes are enclosed in a cylindrical copper box, or are wrapped in pairs in paper (the wrapped dishes may as further protection be placed in a biscuit tin), sterilised in the dry oven, and subsequently stored until needed.

Glass slides (3 inches by 1 inch), circular cover-slips (¾ inch, No. 1) and rectangular cover-slips (2 inches by ¾ inch) are required, and are best kept stored in 50 per cent. alcohol. They can usually be cleaned with hot soapy water or soda ash solution; obstinate cases may be placed in chromic acid solution (6 grms. sulphuric acid, 6 grms. potassium bichromate, and 100 ml. water).

Inoculating Loops and Needles.—These are short lengths of platinum wire fused into glass rods, or, more cheaply, of

nichrome wire fixed by a small chuck screw into brass or aluminium holders. A selection of several shapes and sizes is required—*e.g.*, a stout wire with the end slightly flattened, a longer and thinner wire, and two with loops of different sizes. A loop is conveniently formed by winding the tip of the wire round a suitable portion of the tapering point of a propelling pencil; the gap must be closed or the loop will not retain drops of liquid. An inoculating needle or loop is sterilised by heating to redness in the Bunsen flame, the wire being then allowed to cool for a few seconds before it is used to transfer any living organism.

Cotton-Wool.—The use of cotton " wool " for plugging tubes and flasks solves the problem of stoppering vessels that have to be kept free from infection and yet allow access of air to their contents. Plugs should fit moderately tightly; they should enter about 1 inch into the tube or small flask, and should project by nearly the same amount. A well-fitting plug allows free passage of air, but air-borne spores settling on the plug become entangled in the cotton hairs and do not penetrate inside the vessel. Should the plugs become damp, however, spores on or in the plug may germinate, and infection spread to the contents of the vessel. Hence it is essential that plugs do not become damp during or after sterilisation, and media in storage should have free access of air. When media are being sterilised, the plugs should be loosely covered with grease-proof paper in order to prevent condensation water dropping on them. Old plugs, if not dirty or soiled with culture media, may be used again.

Cotton-wool for plugging purposes should be of the non-absorbent type, made of bleached cotton of good quality and free from dusty short fibres. It may be obtained dyed in various colours, and a supply of coloured plugs is sometimes useful for distinguishing different media, etc., although white is to be preferred whenever possible.

Absorbent cotton-wool should not be used for plugging, but is very useful for the filtration of media. Its different frictional properties give it a characteristic snow-like feel that enables one to distinguish it readily from the non-absorbent variety; as its name implies, it wets out readily with water.

Miscellaneous.—Wire baskets for holding test tubes should be padded at the bottom with old cotton-wool. Tubes to be

sterilised in the autoclave, however, should be put into baskets with no cotton-wool, or the wet cotton soon leads to rusting of the bottom of the basket. Rustless baskets may readily be made from perforated zinc sheet and copper wire (a rectangular piece of the zinc being bent into a cylinder and a disc of the same material wired on at one end). Since one never seems to possess sufficient baskets, the collection of household empty tins of a suitable size is a useful habit. Glass " sweet jars " are also cheap and useful for the storage of media and cultures.

A few slide holders are a convenience. These are usually made of stiff wire, but an ordinary spring-clip wooden clothes peg makes a very useful substitute.

A large enamel mug, or a cylindrical vessel of copper or aluminium, serves to contain water in which tubes of agar or gelatin media may be melted as required, the vessel being heated directly over a Bunsen. Grease pencils for writing on glass are obtainable in various colours. A useful hot plate of varying temperature may be made by bending a flat strip of copper into an S-shape; the projecting end is heated by a small Bunsen burner, or by a micro-burner made from a glass T-piece (with one arm drawn to a jet and the other arm sealed and supported in a rubber bung) connected with the gas supply.

A hand centrifuge taking tapered tubes is frequently necessary, especially for concentrating suspended micro-organisms prior to examination, as in the case of milk.

For stains, mounting media, etc., a liberal supply of dropping bottles should be available. The best type for most purposes is that in which the stopper is prolonged into a solid glass rod which reaches almost to the bottom of the bottle.

For handling material to be examined, a few needles mounted in metal or wooden handles, forceps both large and small, a hand lens or a dissecting microscope, and a fine pair of scissors are needed. Razors for section cutting should be flat ground on one side; much can be done with a safety-razor blade. For serious section cutting a microtome is necessary, and here also many workers now replace the knife by an attachment to take safety-razor blades, thus avoiding the tedious business of honing and stropping.

If much drawing has to be done a camera lucida for attachment to the microscope is a convenience, although micro-

photographs are to be preferred whenever possible. Slide preparations may be kept in small boxes holding a number of shallow wooden trays.

Incubators.—Of the larger and more expensive pieces of apparatus, the most essential are incubators and a good-sized autoclave; the latter will be dealt with in the next section. The purpose of incubators is to provide standard temperature conditions for the growth of organisms, such temperatures being above or below the normal room temperature. Incubators are of all types, the waistcoat pocket being a simple blood-heat incubator that should not be forgotten in an emergency. Laboratory incubators are generally electrically controlled—efficient gas and oil types are also available—and require little attention when once adjusted. The contact points of electric incubators should be kept clean, or sticking may occur. In damp climates, if the incubator is to be left switched off, the wire resistances should be removed and stored in a dry place, or they will deteriorate.

The optimum temperature of many bacteria is blood heat, or 37° C.; other bacteria grow better at a lower temperature, and for these an incubator at 22° is usually employed. In the tropics an ice chest or refrigerator is essential, and one is desirable even in temperate climates. For fungi, 25° C. is a safe general temperature, though for many species the optimum temperature is considerably higher. Certain bacteria (thermophilic) grow well at the surprisingly high temperature of 65° C.

Sterilisation.

Media and glassware which are to be used for pure culture work require to be sterilised. Empty glassware is usually sterilised by dry heat in a gas or electric oven; the time depends on the nature of the material, and on the rate of cooling of the oven after the source of heat is removed, but in general it is sufficient to raise the temperature slowly to 160° C., to maintain this for one hour, and then to allow the glassware to cool slowly to 50° C. or less before opening the oven door. The material should rest on asbestos sheeting and not be in direct contact with the metal parts of the oven; otherwise paper wrappings and cotton plugs will be in danger of charring.

Damp sterilisation is more effective, and lower temperatures can therefore be used. Most material can be satisfactorily sterilised by intermittent or "fractional" sterilisation at 100° C. This may be done in any form of steamer, the simplest form—and one capable of useful service in the small laboratory—being an ordinary domestic vegetable steamer. A larger steamer—*e.g.*, Arnold Steriliser—is desirable, with some device for condensing the steam and returning the water to be re-heated. This obviates both the necessity for frequent filling and the inconvenience of steam escaping into the laboratory atmosphere.

If the material to be sterilised contains resistant bacterial spores, some of these may withstand a single exposure to 100° C. But if it is raised to 100° C. for thirty minutes to one hour by steaming on each of three consecutive days, sterility is secured. The mechanism of this is still a debatable point, but the fact remains that the spores are killed off, and the method is variously referred to as fractional, intermittent, or discontinuous sterilisation.

Pressure sterilisation is carried out in an *autoclave*, of which the simplest type is again well known to the housewife in the form of the "pressure cooker." The usual laboratory form is a copper vessel containing water (heated from below by means of a gas ring), above which is a perforated container for holding the material to be sterilised. The lid can be securely bolted down, and is fitted with an outlet tap, a safety valve, and a pressure gauge. When using the autoclave it is essential to wait until steam is issuing freely before closing the outlet. When the desired pressure is reached, it is maintained by lowering the gas and adjusting the safety valve in such a manner that there is a gentle issue of steam from the valve at this pressure. The lid should be fitted with a composition washer, and the life of this is prolonged if the lid is not screwed down more tightly than is necessary to prevent leakage of steam.

Pressure must not be allowed to fall rapidly, or any liquid medium present will boil up under the reduced pressure and may wet the cotton plugs, if not blow them out altogether. Most media are sterilised by one treatment of half an hour at 15 lbs. excess pressure; more may be desirable in certain cases. The treatment is dependent on several factors—*e.g.*, the size,

shape and consistency of the material. Thick cereal mashes are particularly difficult to sterilise. The expression " sterilisation at 15 lbs." means sterilisation at 15 lbs. *excess* pressure over the atmospheric pressure—*i.e.*, 15 lbs. on the gauge—but an actual pressure of 30 lbs. This corresponds to a temperature of 121° C.

Pasteurisation is a general term for the process of heating sufficient to destroy vegetative forms, but not spores. It involves raising the temperature of the material to 63°-85° C. (according to circumstances), maintaining at this temperature for fifteen to thirty minutes, and then cooling rapidly. Non-sporing bacteria are killed, but not the spores. Pasteurisation is employed in dairy work (*cf.* Chapter IX.), and in the isolation of spore-forming bacteria—*e.g.*, *Clostridium welchii* —from a mixture containing non-sporing organisms.

Sterilisation by Flaming.—Small objects such as glass slides, inoculating needles, etc., may be sterilised by simply passing them through the Bunsen flame several times. When examining cans of preserved food it is desirable to sterilise the opening instrument and the surface of the can by flaming or by burning methylated spirits on them.

Sterilisation by Antiseptics.—It is a good working rule that all infected slides, pipettes, etc., should be placed in antiseptic solution immediately after use. For this purpose a large jar containing 2 per cent. lysol, 5 per cent. phenol, or 0·1 per cent. mercuric chloride should be kept on the working bench. Further notes on sterilisation by antiseptics will be found in Chapter VIII.

Sterilisation by Filtration.—Bacteria may be removed from liquid cultures without application of heat by sucking the fluid with a vacuum pump through specially prepared filter candles made of unglazed porcelain (Chamberland), baked kieselguhr (Berkefeld or Mandler), or asbestos (Seitz). These filters are of various grades of porosity, the finest holding back all bacteria and even some of the filtrable viruses. For ordinary bacteriological work the Chamberland L2 is very useful. The average pore diameter of such filters is from 2 to 10 μ, which is several times larger than the diameters of small organisms, but physical and electrical forces cause the latter to adhere to the sides of the pores. Some viruses may be retained by filters made of collodion, and hence may be

separated from others which are not. Enzymes usually pass through filters which are fine enough to prevent the passage of bacteria.

REFERENCES

EYRE, J. W. H. (1930): Bacteriological Technique. London.
JORGENSEN, A. (1939): Micro-organisms and Fermentation. London.
RAWLINS, T. E. (1933): Phytopathological and Botanical Research Methods. London.
SALLE, A. J. (1939): Fundamental Principles of Bacteriology. New York.

CHAPTER V

THE ISOLATION AND EXAMINATION OF MICRO-ORGANISMS

THERE is great variety in the microbiological technique used by different workers. For a detailed description see Eyre (1930), Muir and Ritchie (1937), Rawlins (1933), Hunwicke (1931), Smith (1938), Tanner (1938), and Abderhalden (1921-34). All that can be given here is a brief summary of the more important everyday procedures, skill in which can be acquired only by practice and by watching an experienced worker.

Atmospheric Infection.—The universal distribution of micro-organisms in air, soil, and water is not always realised. In a normal indoor atmosphere bacteria and fungal spores will descend on one's outstretched palm at the rate of several per minute. A rough estimate of the amount of atmospheric contamination may be obtained by exposing petri dishes of sterile nutrient agar with the lids removed for, say, 0, 1, 10, and 30 minutes respectively, and then replacing the lids and incubating the dishes for several days. Most organisms falling into the agar will then have grown into " colonies " large enough to be seen with the unaided eye.

For the laboratory it is desirable to have a clean, still atmosphere, and when sterile vessels have to be opened this should be done with due precautions to avoid infection.

Pathogenicity.—Pathogenic bacteria represent only a small proportion of the total numbers, whilst fungi pathogenic to man are still more rare. It is nevertheless a good rule when working with unknown organisms to regard them as potentially dangerous; old culture tubes, petri dishes, and other glassware should be sterilised in the autoclave before being washed, and all infected slides and pipettes should be placed in 2 per cent. lysol solution.

Transfer of Cultures (Sub-Culturing).—The culture tube should be held in the left hand, horizontally if the medium is

solid, and obliquely if it is liquid. A wire loop or needle held in the right hand is flamed until red hot and allowed to cool, and the plug is then removed between the little finger and palm of the right hand. The loop is then inserted swiftly and without touching the sides, a small portion of the culture is removed, and the plug is replaced. If the transfer is to be made to another tube, this is also held in the left hand parallel with the first tube, and the second plug is removed when the first has been replaced. The end of the loop or needle is drawn once along the surface of the solid medium; if the medium is liquid, the growth should be tapped off on the side of the tube just above the liquid level. For some purposes it is more convenient to make a " stab " culture by thrusting the inoculating needle into medium which has solidified in a vertical tube. The essentials are neat and speedy handling without muddle, and, provided that the atmosphere is reasonably clean, infection seldom occurs during the above operation. The loop or needle should be flamed before it is replaced on the working bench.

Flaming of Plugs.—The cotton plug should be lighted, and the flame allowed to play round the mouth of the tube or flask for a moment. It is then " breathed " out, not blown out with great effort. The object of this practice, which is frequently overdone, is to prevent infection on the glass or cotton from falling into the flask when the plug is withdrawn. The flaming of plugs *after* they have been replaced is useless as an aseptic precaution, though sometimes useful for distinguishing tubes.

Preparation and Examination of Slides.—" Fixation " of cell structures as applied to botanical specimens is not usually necessary for the examination of bacteria; nor is albumen needed to make them adhere to the slide whilst being stained. All that is required is to spread a small drop of the liquid bacterial suspension on a grease-free slide, allow to air dry, and with the bacterial film uppermost pass quickly through the Bunsen flame several times. The suspension is either a small drop of a liquid culture, or is obtained by emulsifying a trace of the bacterial growth on a solid medium in a small drop of distilled water placed on the slide. The film may be stained by flooding with dilute carbol fuchsin or methylene blue, warming if necessary; it is then washed, dried, and

examined direct under the microscope. No cover-glass is necessary.

It is advisable to examine the preparation first under a low power, focussing on a group of stained organisms in the centre of the field. The barrel of the microscope is then raised, a drop of cedar-wood oil placed in position on the slide, and the $\frac{1}{12}$ inch objective carefully lowered into the oil until it almost touches the slide. The condenser is raised and the microscope barrel is racked up, using the coarse adjustment, until the slide is in focus, when the fine adjustment may be used.

An eyepiece micrometer should always be available, so that the size of the objects under examination may be calculated.

An excellent book on the methods of microscopy is that of Langeron (1934).

"Hanging Drop" Slides.—A slide with a central concave depression is required. A drop of bacterial suspension is placed at the centre of a clean cover-glass, which is then inverted over the cavity and kept in position by a trace of vaseline or immersion oil. Such preparations are of value when examining bacteria for motility. It is best to focus first with a low power on the edge of the drop. A $\frac{2}{3}$ inch objective and a 20x eyepiece usually suffices to detect motility, and a trace of Indian ink or nigrosin added to the drop assists to show up the bacteria.

Preparations in a Mounting Medium.—Large objects, such as fungal structures, may be mounted under a cover-slip in a drop of lactophenol (see Chapter VI.). This is an excellent mounting medium of suitable refractive index, and obviates any trouble due to the formation of air bubbles. If the lactophenol contains 0·05 to 0·08 per cent. of cotton blue, fungal hyphæ will absorb the stain and be more clearly distinguishable.

Sealing Compositions.—Permanent preparations in Canada balsam are somewhat troublesome to make, and the colour of the stain is apt to fade. Slides mounted in lactophenol may conveniently be made semi-permanent by painting round the edge of the cover-slip with a mixture made by warming together equal parts of vaseline and paraffin wax. A rather better sealing preparation has the composition: resin, 80 parts; lanoline, 20 parts, or a solution of shellac in alcohol may be used.

Isolation of Pure Cultures.—Isolation of organisms and

estimation of total numbers is carried out by means of " plate cultures " of suitable dilutions on solid media. The organisms, separated by dilution, are kept separated by the setting of the medium; the individual bacteria, yeasts, or fungal spores then multiply during incubation, and form " colonies " that can be seen, counted, and transferred to sterile culture tubes if required. Some colonies are, of course, derived from clumps of bacteria that have failed to separate; hence when a pure culture is required it is safer to carry out several successive platings before selecting a final colony for sub-culture.

When making the dilutions, a number of test tubes, each containing 9 ml. of sterile saline or other diluent, such as tap water or Ringer's solution (Chapter VI.), are placed in a rack; 1 ml. of the suspension to be plated is then transferred by a sterile pipette to the first tube and thoroughly mixed with the saline either by a rotary shaking of the tube, or by sucking up several times to the 1 ml. mark; 1 ml. of this is then transferred (by a second sterile pipette) to the second tube of diluent, which then contains $\frac{1}{10}$ ml. of the original suspension. Further dilutions to, say, $\frac{1}{1000000}$ are made in the same way. Meanwhile some " stabs " of agar medium have been melted in a water bath and cooled to 45° C.; 1 ml. portions from appropriate diluent tubes, say those containing $\frac{1}{1000}$, $\frac{1}{10000}$, and $\frac{1}{100000}$ of the original suspension, are transferred to separate sterile petri dishes—one sterile pipette will serve so long as the highest dilution is taken first—and a tube of melted agar added to each dish and mixed with the diluent. After cooling, the plates are incubated for several days until the colonies are big enough to be counted. By an appropriate calculation the number of organisms present in each ml. of the original suspension may be ascertained. Actually the plate counts will give results which are too low, since some of the colonies will come from clumps and not from single organisms, and also because certain organisms present may fail to develop on the medium used, or under the aerobic conditions of plate cultures (see later note on anaerobic cultures). Soil, fabric, and other solid materials may be shaken up with diluent under standard conditions, and further dilutions of the resulting suspension can be plated. Methods for *direct* microscopic estimation of the number of bacteria in liquids such as milk are described in Chapter IX.

Moist Cell Cultures.—The development of yeasts, etc., may be watched in a hanging drop culture (see above). It is more convenient to make a larger cell by vaselining a small glass ring with ground edges on to the slide, and placing on this a cover-slip, on the lower surface of which has been placed a smear of agar medium inoculated with the organism to be observed. A drop of sterile water is placed in the moist chamber to prevent drying up, and the cover-slip is sealed with vaseline. Even in such a chamber the air supply may be insufficient for normal fungal development, and it is often better to make the cultures on films of agar medium on glass slides—or on slips of sterile cellophane soaked in liquid medium—kept moist in sterile petri dishes. For the examination of delicate structures which are destroyed on handling the following method is extremely valuable:

Agar Split-Disc Method.—This technique, which has been described by Vernon (1931), is excellent for the examination of sporing heads of the mould fungi. The sporing heads are caused to grow horizontally instead of vertically, projecting into an air channel passing below the cover-slip. A preparation is made by placing three drops of a suitable agar medium —*e.g.*, Czapek's—by means of a sterile pipette and when still hot, on a flamed slide. As the agar begins to gel, a flamed circular cover-slip is lowered on to it so that it flattens out and forms a thin disc. The stage of cooling at which the cover-slip should be lowered into position is roughly that at which the agar ceases to flow when the slide is tilted.

After the disc has cooled (in a sterile petri dish) the cover-glass is made to slide off, a cut is made across the disc with a flamed razor blade, and the two halves are separated by about $\frac{1}{4}$ inch. The half-discs are inoculated with a few spores of the fungus and the cover-glass is replaced. The above steps are, of course, carried out with the usual aseptic precautions. The preparation is then incubated at appropriate temperature in a petri dish containing a little damp blotting paper to secure a moist atmosphere. The sporing heads can be microscopically examined directly (after allowing several minutes for the moisture of condensation to evaporate) at all stages of growth, without requiring any manipulation. Such a culture is, of course, not proof against infection, but will usually remain pure for several days.

A modification found useful in the writers' experience is to fix and stain the preparation at any desired stage by gently flooding the channel with lactophenol-cotton blue. If this is carefully done, the sporing heads are seldom damaged, and the preparation may be made semi-permanent by ringing with sealing composition (see above).

Cellophane Cultures.—A convenient way to secure preparations of mould fungi with delicate sporing structures is to grow them on half-inch squares of sheet viscose (moisture proofing if present should be removed). The squares are placed in a test tube, covered with water and sterilised. About six are then transferred to a sterile petri dish and a loopful of dilute wort containing spores is placed on each. The petri dish is incubated, and at appropriate intervals a square is withdrawn and mounted in lactophenol under a cover-slip for examination.

Indian Ink Methods.—A fine quality Indian ink, diluted 5 to 10 times, is often used as a negative stain or dark background for bacteria or yeasts. A saturated solution of nigrosin gives even better results. A drop of the bacterial suspension is mixed on a slide with a drop of the Indian ink spread over a suitable area, and allowed to dry without heating. It is then examined direct, the bacteria showing colourless against a dark background, like glass rods over which has been scattered a layer of black sand. The effect is very much the same as that produced by genuine "dark ground illumination" by lateral light, which requires a special condenser. This method of negative staining is useful where bacteria are to be measured, since there is less distortion than with a stained preparation.

Single Spore Isolations.—Indian ink is also used in certain methods of obtaining cultures from single spores. The various methods of single spore isolation are reviewed by Rawlins (1933).

In general, however, the safest and best method of obtaining a pure culture is to "plate out" several times in succession, each time selecting a well-isolated colony for further purification. In any case, other methods of single spore isolation should, whenever possible, be followed by plating according to the dilution technique.

Anaerobic Cultures.—All fungi and most bacteria grow aerobically. Some bacteria, however, will not tolerate the

presence of oxygen, and are termed *anaerobes*. Such types will usually grow in deep tubes of medium—recently boiled to expel dissolved air—conditions then being sufficiently anaerobic below the top inch or so of medium. Anaerobic bacteria may be plated out in petri dishes provided that these are incubated in the absence of air—*e.g.*, by placing them in a vacuum desiccator, exhausting, and replacing the air by an inert gas, such as nitrogen or hydrogen. An efficient and popular technique is described by McIntosh and Fildes (see Medical Research Council, 1931). Alternatively the oxygen—or the residual oxygen after exhaustion—may be absorbed by pyrogallol solution contained in the desiccator; for each 100 ml. of air space about 1 grm. of pyrogallic acid and 10 ml. of 10 per cent. caustic soda or caustic potash are required.

Many other methods of securing anaerobic cultures are described in the textbooks. One very useful method not usually given is to replace the petri dishes in the plating-out process by open-ended tubes. These are plugged and sterilised, and rubber stoppers to fit are sterilised separately in a tube of water. The tubes are then fitted with stoppers at the lower ends and the saline suspensions added. Melted and cooled agar medium is then poured in in the usual way. Anaerobic bacteria will develop in the lower part of the agar, whilst the upper half-inch of medium must be neglected as being in contact with air. By removing the stopper and allowing the agar cylinder to slide out of the tube for some distance, thin wafers containing a single colony may be cut off with a flamed knife (reflamed for each cut), and the colonies can then be subcultured.

Special Diagnostic Reactions.

Detection of Acid and Gas Formation.—One of the most important diagnostic characters for bacteria, yeasts and certain fungi is the way in which different sugars are fermented. A 1 per cent. or 0·5 per cent. solution of the sugar is used, in neutral broth or peptone water tinted with litmus or other indicator (see Chapter VI.). Acid formation, if it occurs, is indicated by reddening of the litmus. Gas formation, if slow, may not be obvious, and the simplest method of detecting it is to have a small inverted tube (Durham tube or small ignition tube) in each tube of sugar medium. During sterilisa-

tion the air is expelled from the small tube, and during subsequent growth of the organism this tube acts as a bell jar, and any gas collected in it is readily visible.

Other useful diagnostic tests are for ability to liquefy gelatin (see p. 60), reduce nitrates (see below), reduce sulphates (see p. 23), produce indole (see below), hydrolyse starch (see p. 47), or to evolve ammonia or hydrogen sulphide.

The reduction of nitrates to nitrites is detected by the formation of a red colour in 10 ml. nitrate peptone water culture (see p. 56) upon addition of 2 ml. of a mixture of equal parts of (*a*) sulphanilic acid 0·5 grm., distilled water 100 ml., glacial acetic acid 50 ml.; and (*b*) 0·1 grm. *a*-naphthylamine dissolved in 20 ml. warm water, and added to a mixture of 100 ml. water and 50 ml. glacial acetic acid. Solutions (*a*) and (*b*) are kept separate. Control tests are essential, including a test for ammonia on a similar culture with Nessler solution, in case the organism reduces the nitrite as soon as formed.

The reactions for differentiating members of the *coli-aerogenes* group are important, and are as follows:

A drop of the culture liquid—*e.g.*, the last MacConkey or bile salt lactose broth showing acid and gas development—is smeared by means of a sterile bent glass rod on the surface of dry MacConkey agar plates. Red colonies developing at 37° C. are transferred to peptone water, and subsequent growth is used as the inoculum for the following tests (see also Chapters VI. and XII.):

Indole Test.—A three- to seven-day culture in peptone water is shaken with 1 ml. ether, allowed to stand two minutes, and the dissolved indole, if any, in the ether layer is tested with a few drops of Ehrlich's reagent (paradimethylamidobenzaldehyde 4 grms., alcohol 380 ml., conc. hydrochloric acid 80 ml.). The pink colour of rosindole indicates a positive reaction.

Methyl Red Test.—Dextrose phosphate broth is inoculated and incubated for three days at 37° C. Five drops of 0·04 per cent. methyl red solution are added. A magenta colour denotes a positive, a yellow one a negative, reaction.

Voges-Proskauer Test.—Dextrose phosphate broth inoculated and incubated as for the methyl red test is treated with 5 ml. of 10 per cent. sodium hydroxide solution. The tube is left

1. Petri Dish Culture, showing "Colonies" of Yeast. ($\times \frac{2}{3}$.)
2. Stained Preparation of *Bac. megatherium*. ($\times$ 600.)
3. Nigrosin Preparation of *Bac. megatherium*. ($\times$ 600.)

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to stand twenty-four hours at room temperature. A pink fluorescence due to the production of acetyl-methyl-carbinol indicates a positive reaction. In O'Meara's modification of this test a trace of creatine, followed by 5 ml. of 40 per cent. caustic soda, is added. A pink colour, developing within a few minutes after shaking, is positive.

Citrate Test.—Citrate medium is inoculated, using a straight platinum wire, and incubated for one to two days at 37° C. Opacity (cloudiness) denotes a positive reaction.

Detection of Enzymes.—In addition to the various enzymic reactions—such as ability to liquefy gelatin—that are used as diagnostic characters, certain other enzymes produced by micro-organisms may require study for particular purposes.

Active diastase (starch-liquefying) preparations may be made by growing *Aspergillus oryzæ* or other suitable organism on sterile damp bran in the manner recommended by Oshima and Church (1923): 10 grms. of bran moistened with 10 ml. water and sterilised under pressure in a 250 ml. conical flask is inoculated with the fungus, incubated, and the mass extracted with cold water several days later, when it is permeated with the fungal mycelium. The activity of the extract may be tested by adding a few drops to a potato starch paste and maintaining at 60° C. until liquefaction is complete.

Starch liquefying organisms, grown in a petri-dish on agar medium containing starch, will form a clear zone round the colonies; this shows up more clearly if the medium is flooded with dilute iodine. Cellulose fermenting organisms will also develop clear zones in a medium containing precipitated cellulose, but this is troublesome to make up, and it is generally simpler to test their action on filter paper strips half immersed in a suitable nutrient solution deficient in carbon source—*e.g.*, sodium nitrate 0·5 grm., potassium hydrogen phosphate 1 grm., magnesium sulphate 0·5 grm., potassium chloride 0·5 grm., and ferrous sulphate 0·01 grm.; distilled water 1,000 ml.

Lipases may be detected by the clearing of, and acidity produced in, olive oil emulsions. Pectinase may be tested on pectin suspensions, or by its power of disintegrating thin discs of potato. For fuller details concerning the testing and utilisation of enzymes see Waksman and Davison (1926).

The effect of antiseptics on enzyme action is discussed in Chapters VII. and VIII.

Thermal Death Point.—It is often desirable to know the resistance to heat of bacteria or mould spores. This may be done by heating broth suspensions of the organism to an appropriate range of temperatures in a water bath for a suitable time, cooling, and incubating. The period of heating and cooling should be as short as possible, and for more exact work it is preferable to heat the organisms in sealed capillary tubes clipped to the thermometer bulb, the contents being afterwards transferred to a suitable medium and their viability tested.

Care and Storage of Cultures.—A culture may die off owing to: (1) exhaustion of the food supply; (2) poisoning (staling) by the products of metabolism; (3) drying up of the medium; (4) infection by other organisms; (5) exposure to heat, chemical vapours, etc.

Slope cultures on agar and gelatin require to be sub-cultured at intervals varying from a week to a year according to the nature of the organism and the temperature and humidity at which it is stored. In general, cold storage prolongs the life of cultures, and reduces the rate of evaporation.

One of the worst enemies of stored cultures is infection by *mites*, so small that they are only observed with difficulty with the aid of a hand lens. Their attack is often not suspected until they have wandered from tube to tube, despite cotton plugs, devouring cultures of fungi and conveying infection. An early sign that may lead to detection is the "moth-eaten" appearance of the cultures. Small-spored moulds of the *Cephalosporium* and *Sporotrichum* type are often spread by the wandering mites. Mite attack may be prevented by poisoning the plugs, but this method involves obvious dangers as regards the culture. A simpler method is to store the tubes overnight in a closed jar containing a little pyridine or carbon tetrachloride.

Sealed Cultures.—Sealing culture tubes with rubber caps is a bad practice; it certainly delays drying up of the culture, but increases the risk of infection, since the plug becomes damp. A better method is to dip the plugged end of the tube into melted paraffin wax. Any form of sealing, however, leads to restriction of the air supply and concentration of the toxic products of metabolism, and should be avoided when possible.

Tubes containing liquid cultures of bacteria may be sealed

in a blowpipe, and usually remain viable for a considerable time. If acid is likely to be formed the tubes should contain sufficient chalk to neutralise this. Spores of bacteria and fungi may often be preserved for years in sand. The sand is first treated with warm hydrochloric acid, washed, and ignited; or specially prepared sand may be used. Plugged test tubes each containing a few grammes of the purified sand are sterilised in the dry oven, several drops of a spore suspension are added and allowed to dry off in the incubator, and finally the tubes are sealed in a blowpipe. (Thaysen, 1924, 1934; Galloway, 1936.)

Cultures preserved in a dry state by the above method usually retain their biochemical properties for a much longer period than when maintained by sub-culture on ordinary media. Other methods recommended are drying in a frozen state, evacuating, and sealing or drying *in vacuo* over phosphorus pentoxide (Microbiol. Congress, 1936).

Purification of Cultures.—Sometimes the dilution plating technique is inadequate to separate a mixture of organisms, and special methods must be adopted. Pasteurisation usually leaves bacterial spores with unimpaired vitality, whilst killing off vegetative forms; many spores will even survive boiling. Fungal spores rarely survive even a few minutes at 80° C., in the presence of moisture. The ascospores of the mould fungus *Byssochlamys fulva* can resist a rather higher temperature, with the result that this is one of the few moulds giving trouble in the canning industry.

Fungi have a wide pH tolerance, but prefer an acid medium, and certain species, such as *Aspergillus niger*, will grow under very acid conditions. Hence fungi can often be separated from bacteria by growth on an acid medium. Another point of difference is that fungi can utilise ammonium salts and nitrates, whereas most bacteria require organic nitrogen. These two points of difference may be made use of when it is desired to prevent bacterial growth in platings for the isolation of soil fungi—for example, by employing as a medium Czapek's agar (containing nitrogen in the form of nitrate), and acidifying the medium at the time of pouring the plates with 0·5 ml. of 12 per cent. lactic acid per 100 ml. of medium. *Mucor* spp., however, do not grow well on Czapek's agar.

Many other devices may be utilised in special instances.

A rapid growing organism may be allowed to grow away from a slow growing contamination; the difference in resistance to incubation temperature, to mild antiseptics, or to the osmotic state of the medium may be used; or special enrichment media may be used to increase the proportion of the desirable organism. Bacterial contamination of a fungal culture growing on agar may often be removed by cutting out and inverting a portion of the agar, and letting the fungal hyphæ grow through.

Section Cutting.—For the microscopic examination of certain materials it is necessary to cut thin sections. For most purposes hand sections, cut with an ordinary razor, a section-cutting razor with one side flat ground, or even with a safety razor blade, will be found sufficient. The object if small may be supported in pith or in fresh carrot whilst being cut.

For serial sections a microtome of the Cambridge Rocker type is satisfactory. A detailed description of the technique of microtome work is given by Chamberlain (1932) and Rawlins (1933).

Microchemistry and Staining Reactions.—Some acquaintance with the methods for the detection of small quantities of chemical substances such as starch, sugars, cellulose, proteins, fats, alcohol, acetone, etc., is useful to the microbiologist. Summaries and further references to the literature are given by Garner (1932) and by Rawlins (1933).

Agricultural Microbiology.—For an account of the special technique connected with the study of soil bacteria, reference may be made to the small manual of Fred and Waksman (1928) and Cunningham (1934).

Methods for the isolation of plant disease organisms and for the artificial inoculation of plants with pathogenic bacteria, fungi and viruses are summarised by Rawlins (1933).

REFERENCES

ABDERHALDEN, E. (1921-1934): Handbuch der biologischen Arbeitsmethoden.
CHAMBERLAIN, C. J. (1932): Methods in Plant Histology. Chicago.
CUNNINGHAM, A. (1934): Practical Bacteriology. London.
ELLIS, D. (1923): Practical Bacteriology for Chemical Students. London.
EYRE, J. W. H. (1930): Bacteriological Technique. London.
FRED, E. B., AND WAKSMAN, S. A. (1928): Laboratory Manual of General Microbiology. New York.
GALLOWAY, L. D. (1936): Ind. J. Agric. Sci., 6, 946.
GARNER, W. (1932): Industrial Microscopy. London.
HUNWICKE, R. F. (1931): Essentials of Bacteriological Technique. London.

JORGENSEN, A. (1939) : Micro-organisms and Fermentation. London.
LANGERON, M. (1934): Précis de Microscopie. Paris.
MACKIE, T. J., AND MCCARTNEY, J. E. (1931): Practical Bacteriology. Edinburgh.
MEDICAL RESEARCH COUNCIL (1931): A System of Bacteriology, **9**, 94. London.
MICROBIOLOGY, INTERN. CONGRESS OF (1936): Report, pp. 40-43.
MUIR, R., AND RITCHIE, J. (1937): Manual of Bacteriology. London.
OSHIMA, K., AND CHURCH, M. B. (1923): Ind. Eng. Chem., **15**, 67.
RAWLINS, T. E. (1933): Phytopathological and Botanical Research Methods. London.
SALLE, A. J. (1939): Fundamental Principles of Bacteriology. New York.
SMITH, G. (1937): Introduction to Industrial Mycology. London.
TANNER, F. W. (1928): Introduction to Bacteriological Technique. New York.
THAYSEN, A. C. (1924): J. Inst. Brewing, **30**, 349.
—— (1934): *Ibid.*, **40**, 469.
VERNON, T. R. (1931): Ann. Bot., **45**, 733.
WAKSMAN, S. A., AND DAVISON, W. C. (1926): Enzymes. London.

CHAPTER VI

CULTURE MEDIA AND STAINS

CULTURE MEDIA

MANY different culture media have been devised. One reason for this is that certain organisms only develop well on particular media. Another reason is that special media are required to bring out certain chemical or physical characters of the organism. It is, however, obviously desirable not to increase the number of media more than necessary, and only a brief selection of the more essential types is given here.

Media may be classified as "natural" and "synthetic." Natural media—*e.g.*, nutrient broth—contain organic matter derived from some animal or vegetable source, and are often best for growth purposes. Thus many organisms grow best on a sterile infusion of their usual substratum. Synthetic media—*e.g.*, Czapek's medium—are prepared from chemical substances obtainable in a state of purity; they are therefore of standard composition, at whatever time and place they are made up, and this is a great advantage for descriptive and diagnostic purposes.

For special purposes "selective" media—*e.g.*, bile salt lactose medium—are employed, designed to favour the development of certain groups of organisms and to suppress others.

In addition to the composition of a medium, its reaction must be considered. Most bacteria develop best at a neutral or slightly alkaline reaction—say, pH 7·5—although, of course, such organisms as the lactic and butyric bacteria can tolerate moderate acidity. Fungi usually tolerate a wider pH range than do the bacteria, but develop best on the acid side—pH 5·0 being a suitable reaction for most types.

It is therefore necessary to adjust the reaction of a medium before sterilisation, and since the heating process frequently causes some change (usually in the direction of increasing acidity), the sterilised medium should be cooled and its reaction

again tested. In special instances further adjustment with sterile acid or alkaline solutions is necessary. When for special purposes a particularly acid or alkaline agar or gelatin medium is required, adjustment should be made after sterilisation, or the medium will fail to set properly.

The older methods of adjusting culture media in terms of standard solutions of acid or alkali have been generally replaced by the use of the pH scale, and adjustment by testing samples with colour indicators, such as phenol red (pH 6·8-8·4) and bromcresol purple (pH 5·2-6·8), is usually quite sufficient. If the medium itself is coloured, a comparator arrangement of the usual type may be used. For details of pH measurement, preparation of buffer solutions, etc., reference may be made to Clark (1928).

Litmus has long been in use as an indicator, and, being non-toxic to organisms, has the advantage that it may be used to tint the medium itself. It is, however, liable to be reduced, and for certain purposes brom-cresol-purple, brom-thymol blue, or neutral red included in the medium has proved superior to litmus.

When media are to be stored in tubes, the tubing should be done before sterilisation, never after it, except when this is unavoidable—*e.g.*, when some addition has to be made subsequent to the heating process—and in such cases great difficulty will be found in securing freedom from infection. After sterilisation and cooling, media should be incubated to test their sterility before they are put into use.

Liquid Media.

Saline.—Distilled water, 1,000 ml.; sodium chloride, 8 grms. This is a dilution medium, not a growth medium. The salt is added to prevent osmosis, and consequent swelling of the organisms.

Dissolve the salt, transfer to tubes by means of a burette, putting 9 ml. in each tube, since this is the amount required in carrying out the dilution technique; plug the tubes and sterilise for twenty minutes at 15 lbs. There is usually some slight loss during sterilisation, and, of course, in storage, so that for ordinary purposes it may be better to put 10 ml. in the tubes, allowing 1 ml. for loss by evaporation. For ac-

curate work 9 ml. of sterile saline must be transferred to sterile tubes just before use; or larger quantities—*e.g.*, 90 ml.—may be used.

After detailed tests on the bacterial effect of various diluents, Wilson *et al.* (1935) recommend sterile tap water or, still better, a diluent containing Ringer's balanced mixture of salts, in preference to saline.

Ringer's Solution has the following composition: sodium chloride, 9·0 grms.; potassium chloride, 0·42 grm.; calcium chloride, 0·48 grm.; sodium bicarbonate, 0·2 grm.; glass-distilled water, 1,000 ml. This is diluted to quarter strength with glass-distilled water and autoclaved at 120° C. for twenty minutes.

Nutrient Broth.—Water, 1,000 ml.; sodium chloride, 5 grms.; peptone, 10 grms.; meat extract, 5 grms. "Lemco" has long been regarded as the standard, but other meat preparations, or vegetable extracts such as "Marmite" or "Yeastrel," are also available. Dissolve, adjust reaction to pH 7·5 with dilute caustic soda solution, steam for one hour, filter through a pleated, thick filter paper, then tube, plug with non-absorbent cotton-wool and sterilise at 15 lbs. for twenty minutes. This is a good medium for bacteria. For testing antiseptics by the Rideal-Walker method use 20 grms. "Lab-Lemco," 20 grms. peptone, and 10 grms. salt per litre of water. A clear solution is essential.

Wort.—To 250 grms. of freshly ground malt (a low dried malt with a high diastatic power) add 1 litre water at 65° C. Maintain at 60°-65° C., but not exceeding this, on a water bath for an hour or so, with occasional stirring. Remove when the residue of the malt on testing with iodine ceases to give the reaction for starch. Strain through muslin, autoclave at 5 lbs. for ten minutes, cool and filter. Tube and steam sterilise for thirty minutes on each of three consecutive days. The solution will contain about 12 per cent. maltose and does not require neutralisation, as the medium should remain slightly acid. This medium is excellent for the growth of fungi and yeasts.

Instead of making up the wort in the laboratory, it is sometimes convenient to obtain unhopped wort from the brewery as required, or to use diluted malt extract.

Czapek's Solution (Dox's Modification).—Distilled water,

1,000 ml.; saccharose, 30 grms.; sodium nitrate, 2 grms.; potassium dihydrogen phosphate, 1 grm.; potassium chloride, 0·5 grm.; magnesium sulphate, 0·5 grm.; ferrous sulphate, 0·01 grm. Dissolve the ingredients separately, tube, and sterilise as for wort. This medium is more particularly for fungi and is a "synthetic" medium. Some fungi—*e.g.*, the Mucors—and most bacteria do not grow well in it.

Peptone Water.—Distilled water, 1,000 ml.; sodium chloride, 5 grms.; peptone, 10 grms. Steam, adjust to pH 7·5, steam and filter. Tube, plug and autoclave for twenty minutes at 15 lbs. This medium is used for the indole reaction.

Litmus Milk.—Add sufficient litmus solution to separated new milk to give a bluish-purple colour. Tube, and steam sterilise for thirty minutes on three or more successive days. In place of litmus the addition of 10 ml. of 0·5 per cent. solution of brom-cresol-purple to 1,000 ml. separated milk provides a useful indicator. Either of these is a useful medium for determining milk curdling, peptonisation and gas production in milk.

Sugar Broths.—These are used for determining fermentation reactions (production of acid and gas) as an aid to identification of micro organisms. Nutrient broth containing 1 per cent. dextrose, maltose, lactose, or other sugar under test, plus a colour indicator such as litmus, are filled into test tubes containing inverted smaller tubes (see p. 45) to detect gas production.

Various modifications are used by different workers. Some prefer to have the sugar in 0·5 per cent. concentration or even less. It must be remembered, however, that many organisms forming acids from sugar will also form alkali from the peptone in the medium, and the latter effect must not be allowed to mask the former. Other indicators than litmus are sometimes used, or the indicator is omitted altogether and colorimetric tests made on samples before and after incubation. A useful indicator is Andrade's, prepared by dissolving 0·5 grm. acid fuchsin in 100 ml. distilled water and adding 17 ml. of normal caustic soda solution. One per cent. of the indicator is added to peptone water of pH 6·8, and after autoclaving a steam sterilised solution of the specific sugar is added aseptically, and the medium tubed as above. When acid, the medium is magenta red.

For sterilising sugar broths Davis (1938) recommends momentary autoclaving. This is effected by regulating the heating of the autoclave so as to take twenty minutes to reach 120° C., then turning off the gas and allowing the autoclave to cool.

Dextrose Phosphate Broth (for methyl-red and Voges-Proskauer tests).—Distilled water, 1,000 ml.; peptone, 5 grms.; dipotassium hydrogen phosphate, 5 grms. Steam, adjust and filter as for peptone water. Add 5 grms. dextrose, tube and autoclave at 10 lbs. for ten minutes.

Citrate Medium (for differentiation of *Bact. coli* and *Bact. aerogenes*).—Distilled water, 1,000 ml.; sodium chloride, 5 grms.; magnesium sulphate, 0·2 grm.; ammonium dihydrogen phosphate, 1 grm.; dipotassium hydrogen phosphate, 1 grm. Dissolve, add 2 grms. citric acid and adjust the reaction to pH 6·8 with normal caustic soda. Tube, autoclave 15 lbs. for ten minutes.

Bile Salt Lactose (MacConkey) Broth (for indicating the presence of *Bact. coli*).—Water, 1,000 ml.; sodium taurocholate, 5 grms.; lactose, 10 grms.; peptone, 20 grms.; sodium chloride, 5 grms. Dissolve by steaming for one to two hours, cool, filter, adjust to pH 7·4, using phenol red. Add about 10 ml. of 1 per cent. neutral red solution, tube in 5 ml. quantities with Durham tubes. Autoclave at 10 lbs. for fifteen minutes, or steam sterilise on three days.

Sulphate Reduction Medium.—Distilled water, 1,000 ml.; dipotassium hydrogen phosphate, 0·5 grm.; ammonium chloride, 1 grm.; calcium sulphate, 1 grm.; magnesium sulphate, 2 grms.; sodium lactate, 3·5 grms.; ferrous ammonium sulphate, 0·5 grm. Agar may be added to make a solid medium.

Nitrate Peptone Water (for reduction of nitrates to nitrites).—Dissolve 5 grms. nitrite-free potassium nitrate in 1,000 ml. peptone water (see p. 46). Tube and sterilise at 15 lbs.

Agar Media.

Agar, obtained from a seaweed, is a condensation form of the hexose galactose; 1½ to 2 per cent. of agar, either shred or powder, gives the necessary setting power to a medium. The agar is dissolved by heating with the medium for half an hour or more preferably at several pounds pressure, and the medium is then filtered through a thin layer of damp absorbent cotton-

wool. It is then run into test tubes by means of a burette or dropping funnel (warmed before use) and the tubes plugged and sterilised in the usual way. During filtration the medium should be kept hot. Instead of absorbent cotton-wool (which may be supported on cheese cloth if desired), special coarse filter paper can be obtained for this purpose. Or the hot agar may be filtered by suction through a Buchner funnel containing a filter medium of pulp. The latter is prepared by placing an ordinary filter paper on the funnel, followed by a hot mash of finely rubbed filter paper pulp. When sucked down the pulp should be 1·5 mm. thick. Eject the water and pour on the hot agar medium, breaking its fall by a small piece of stiff paper placed on the pulp.

Albumen is sometimes used for clarifying agar (or gelatin) media. The white of one egg, or 10 grms. dried albumen ground finely with a little water, is stirred into 1,000 ml. of the melted agar medium at 50°-60° C. and shaken vigorously. The medium is then steamed for an hour, or heated for half an hour at 5 lbs. pressure, and allowed to cool to 50°-60° C. The coagulated albumen settles, taking fine particles with it, and the supernatant fluid is then filtered through pulp or absorbent cotton-wool, tubed and sterilised.

It is usually convenient to adjust the pH before addition of agar and to check again after melting.

Agar media are usually tubed as "stabs" or "slopes." The former are prepared by filling the tubes about half full; the latter by filling the tubes one-third full, and after the final sterilisation allowing the agar to solidify when the tubes are in an inclined position. For this purpose a specially constructed board may be used, or if this is not available the inverted lid of a long cardboard box is quite useful.

A special feature of agar is that it emits water vapour on setting. For this reason petri dishes containing agar should be inverted as soon as set, and incubated in this position. Otherwise the surface of the agar is wet, and drops of condensed water from the glass lid splash amongst the developing colonies.

Agar jelly sets at about 40° C., but melts only if heated to 96° C.

Ordinary agar contains soluble impurities, and when required for special purposes the shred form should be employed and

washed in running water for twelve to twenty-four hours before use.

Nutrient Agar.—This is simply nutrient broth with the addition of 1½ per cent. agar. The pH should be 7·5-8·0 before sterilisation and 7·0-7·5 after sterilisation.

Wort Agar.—Wort made as described (and diluted if desired), plus 2 per cent. agar, is sterilised on three consecutive days at 100° C. This is an excellent medium for fungi, and if it is desired to suppress most bacteria the maltose content of the wort should not fall below 5 per cent.; the increased amount of agar is added, and pressure sterilisation avoided, on account of the slight acidity of the medium. Malt is not always readily available, and instead of wort in the above medium a 5 per cent. (or stronger) solution of malt extract may be used.

The addition of 3 per cent. powdered chalk is recommended if the medium is to be used for the maintenance of stock cultures.

Czapek's Agar (Dox's modification).—To Czapek's solution add 1½ per cent. agar, and adjust to pH 7·5 after melting. This is one of the standard media for fungi, but does not always produce such a vigorous growth as wort agar.

Where a medium is desired suitable for growth of fungi, but not for bacteria, Czapek's agar may be used with the addition—after melting and just before use—of a 12·5 per cent. solution of lactic acid at the rate of 0·5 ml. to every 100 grms. of agar medium. This gives sufficient acidity to check the growth of most bacteria.

Dextrose Agar, Lactose Agar, etc.—These are as nutrient agar with the addition of 1 per cent. of the pure sugar. They should be given fractional and not pressure sterilisation.

Milk Agar for Milk Counts.—Yeastrel, 3 grms.; peptone, 5 grms.; washed shredded agar, 15 grms.; fresh whole milk, 10 ml.; distilled water, 1,000 ml. Dissolve the Yeastrel and peptone in distilled water in the steamer and adjust to pH 7·4 at room temperature, using phenol red as indicator. Wrap the agar in muslin and wash in running cold water for fifteen minutes. Squeeze out excess water and add the agar and freshly shaken milk to the broth, autoclave at 15 lbs. for twenty minutes. Filter hot through pulp, adjust if necessary the pH to 7·0, tube and autoclave at 15 lbs. for twenty minutes.

Bile Salt Lactose Agar.—First make bile salt lactose broth

(p. 56), omitting the lactose. Add 2 per cent. agar, adjust the reaction to pH 7·5, steam and filter, and add 10 grms. lactose and about 10 ml. of 1 per cent. neutral red solution. Pour into tubes or flasks and autoclave at not more than 10 lbs. for fifteen minutes.

Starch Agar should contain 1 per cent. potato starch. The starch should be made into a smooth paste with a little cold water, and added—with constant stirring—to nutrient broth, Czapek's or any other suitable nutrient solution as this is brought from 60° C. to boiling point. The agar may then be added in the usual manner.

Oat Agar is favourable for certain fungi. Quaker oats 6 per cent. and agar 2 per cent. are suitable proportions. Many other agar media have been used for fungi—potato, prune, cherry and other infusions being favoured by various workers.

Blood Agar for growing certain animal pathogens and demonstrating the presence of hæmolytic streptococci—*e.g.*, *Strep. mastitidis*. To tubes of melted nutrient agar (2 per cent. agar) cooled to 50° C. add carefully 0·5 to 1 ml. of defibrinated ox blood. Then either inoculate with the material to be tested and pour into petri dishes, or pour first into the dishes and spread the inoculum over the dried surface of the set medium in a series. Colonies of hæmolytic organisms show around them a clear zone of hæmolysis due to the solution of the red blood corpuscles. Edwards (1933) recommends the addition of 2 ml. of 0·1 per cent. crystal violet and 1 grm. of aesculin to every 1,000 ml. of medium, in order to make it more selective for mastitis streptococci.

Wilson-Blair Medium for Cl. welchii.—Melted nutrient agar (3 per cent. agar), 100 ml.; add 20 per cent. anhydrous sodium sulphite 10 ml., 20 per cent. commercial glucose 5 ml., 8 per cent. ferrous sulphate 1 ml. The hot medium is added to an equal quantity of the water under examination in a petri dish.

Thornton's Medium (for soil bacteria).—Dipotassium hydrogen phosphate, 1 grm.; magnesium sulphate, 0·2 grm.; calcium chloride, 0·1 grm.; sodium chloride, 0·1 grm.; ferric chloride, 0·002 grm.; potassium nitrate, 0·5 grm.; asparagine, 0·5 grm.; mannitol, 1 grm.; shred agar (washed), 15 grms.; distilled water, 1,000 ml.; pH 7·4.

Gelatin Media.

Gelatin was the first substance employed for the preparation of jelly media, but on account of its low melting point it has now been replaced by agar for most purposes. For organisms that can be incubated at temperatures not exceeding 20° C. gelatin has certain advantages; it gives a clear medium which sets with a dry surface, has nutrient properties, and by its liquefaction affords an indication of proteolytic activity. Stab inoculations (see p. 40) of certain bacteria show characteristic forms of liquefaction useful as an aid to identification. Gelatin plates are incubated in the normal way, not inverted, and must not be allowed to rise above 20° C. Sterilisation of gelatin media should not be done under pressure, nor should the media be acid, or the setting power will be impaired.

Nutrient Gelatin.—To nutrient broth add 10 to 15 per cent. of good quality sheet gelatin. Melt, adjust to pH 7·5, and filter. Sterilise for twenty minutes at 100° C. on three consecutive days. This is the most commonly used gelatin medium, but many other gelatin media will be found in the literature.

Solid Media.

Potato Slopes.—Sound potatoes are well cleaned, and cylinders cut out with a cork borer of such diameter that they fit the tubes to be used. The cylinders are cut diagonally, washed, and a half portion placed in each tube with the lower, thicker end resting on a little moist cotton-wool. Fractional sterilisation is preferable, and the sterility of the finished medium should be tested by incubation.

Many other natural products may be sterilised—*e.g.*, plant and animal tissues for certain pathogenic organisms, wood blocks for wood-destroying fungi, and similar media which seek to make the pure culture work simulate natural conditions as closely as possible.

STAINING METHODS

Staining of Bacteria.

Since bacteria are minute in size, and for the most part colourless, examination in their natural state presents diffi-

culties. It is therefore usual to stain them with suitable dyes in order to bring out details of their structure and composition, and to aid in identification.

The method of preparing a film for staining is described in Chapter V. To obtain a good film it is desirable to use a young culture of the organism, to use only a very small amount of material, and to avoid transfer of particles of agar or other medium to the film. The usual tendency is to make too thick a film. Stains should be filtered before use.

Simple Stains.

The most useful stains for general purposes are dilute carbol fuchsin and methylene blue. The fixed film should be flooded with stain for about one minute (gently warming if this is necessary to secure adequate staining), washed, and air dried.

Carbol Fuchsin (dilute) is a 1:10 aqueous dilution of the Ziehl-Neelsen formula, which is basic carbol fuchsin, 1 grm.; absolute alcohol, 10 grms.; 5 per cent. phenol solution, 100 ml.

Methylene Blue.—This may be used as a saturated aqueous solution, or as Loeffler's methylene blue, which is made by mixing 30 ml. of a saturated alcoholic solution of the dyestuff with 100 ml. of a 1:10,000 aqueous solution of caustic potash.

Other useful stains, details of which will be found in the textbooks, are gentian violet and carbol thionin.

Stains for Special Purposes.

Gram Stain.—This is a stain to which great importance has been given by medical bacteriologists, who divide bacteria into those which are Gram-negative and those which are Gram-positive, according to whether they are or are not decolorised by alcohol or other decolorising agent after treatment with certain aniline dyes and iodine. In order to show up the organisms which have been decolorised, it is usual to counterstain with a contrasting colour.

The following are two of the many modifications of Gram's stain.

The film to be examined is first stained two to three minutes with carbol gentian violet (1 part saturated alcoholic solution plus 10 parts 5 per cent. aqueous phenol solution). The stain

is drained off, and the film covered with Gram's iodine (iodine 1 grm., potassium iodide 2 grms., distilled water 300 ml.) for one minute. Dry thoroughly by gentle warming or careful blotting. Decolorise with aniline xylol (2 parts aniline to 1 of xylol) until no more stain is removed. Wash with several changes of xylol and dry. Counterstain with dilute carbol fuchsin, wash and dry. The Gram-positive organisms are bluish-purple and the Gram-negative ones red.

Another method is to stain a *thin* film for three minutes with aniline gentian violet, drain, treat with Gram's iodine for two minutes, immerse in methylated spirit or acetone for thirty seconds, wash in water, and counterstain with dilute carbol fuchsin. Aniline gentian violet is made up fresh from two stock solutions: (1) 1 part saturated alcoholic gentian violet, and (2) 10 parts aniline oil water (5 ml. of aniline shaken with 100 ml. water and filtered).

Spore Stain.—Bacterial spores, having a resistant outer wall, do not take stains readily. If, however, they are made to stain by warming for five minutes with Ziehl-Neelsen's carbol fuchsin, cautious treatment with alcohol or dilute sulphuric acid will remove the stain from the vegetative cells, but not from the spores. The vegetative cells may then be lightly counterstained with methylene blue, the spores remaining red. The decolorisation process should be checked at various stages by washing, and controlled by microscopic examination.

An alternative method that gives good results is to stain as above with carbol fuchsin, and then to flood with a film of nigrosin solution and air dry. The spores should show red, and the rods white, against a dark background.

Yet another method is the following: Treat a film from a twenty-four-hour culture with 5 per cent. aqueous malachite green; heat carefully, allowing the stain to steam for ten minutes. Replenish the stain as it evaporates, so that it does not dry on the slide. Allow to cool, wash gently in water, and counterstain with dilute carbol fuchsin or with 5 per cent. aqueous mercurochrome. Spores are stained green, and vegetative (non-sporing) cells red.

Staining of "Acid-Fast" Bacteria.—Certain organisms, and notably the tuberculosis organism, resist decolorisation even more strongly than spores. The film is heated with strong (Ziehl-Neelsen) carbol fuchsin until the stain bubbles, any loss

by evaporation being replaced by the addition of water. Cool, wash, and immerse in 20 per cent. sulphuric acid until no more stain comes away; or decolorise in alcohol containing 1 to 3 per cent. hydrochloric acid. Wash, and counterstain with methylene blue. "Acid-fast" organisms will be coloured red, other organisms blue.

When milk is examined microscopically for tubercle bacteria, it is first centrifuged in a tapered tube, and the film is made from the sediment and stained by the above method. The slide should be examined particularly for animal tissue cells, in which the organisms are more likely to be found, often as red, beaded, faggot-like clusters. Tests involving guinea-pig inoculation with the sediment are essential for diagnosis.

Capsule Stain.—Air dry, but do not fix the film. Stain for two minutes with cold carbol fuchsin and wash gently in water. Cover with a thin layer of nigrosin by spreading a loopful of saturated aqueous nigrosin. Dry quickly to prevent decolorisation of the bacteria. The latter will be red and the capsules white against a blue-grey background.

Flagella Stain.—As the flagella are very fragile and readily detached, great care in handling is required. The film must be thin, and made from either the growth in the water of condensation collected at the base of an eighteen- to twenty-four-hour old agar culture, or from a thin suspension of this young agar culture in a little distilled water, the suspension being incubated for one-half to two hours. Place a drop of the liquid at one end of a perfectly grease-free slide and allow it to run down over the surface. Air dry at room temperature, and place for two to three minutes in a fixing solution of the following composition: potassium bichromate, 2·5 grms.; sodium sulphate, 1 grm.; mercuric chloride, 5 grms.; water, 100 ml. Wash in distilled water and treat for not less than five minutes with the following mordant: 3 parts 20 per cent. tannic acid and 1 part 5 per cent. ferric chloride. Wash again and cover the film with silver solution (see below), and heat gently for one-half to one minute. Wash thoroughly but carefully, holding the slide horizontally and playing the stream of distilled water on one end. Stain with dilute carbol fuchsin for one minute, wash and air dry. The silver solution is made by mixing 5 ml. distilled water with 15 ml. cold saturated aqueous solution of silver sulphate and adding 33 per cent.

ethylamine, drop by drop, until the precipitate first formed is just redissolved.

Negative Staining.—A " dark field " effect resembling that obtained by lateral illumination may be obtained by making a film with a bacterial suspension in Indian ink, saturated aqueous solution of nigrosin, or 2 per cent. aqueous solution of Congo red. The film should be allowed to dry on the slide at air temperature, when the bacteria will show up colourless against a coloured background (see p. 44). If Congo red is used, the film, after drying, should be dipped in acid alcohol (p. 63) to change the red colour to blue.

Newman's Stain for Milk Films.—This stain simplifies the usual procedure of fixing with alcohol, defatting with xylol, and staining with methylene blue. Newman's formula is: (1) Methylene blue, 1 grm.; (2) alcohol, 54 ml.; (3) tetrachlorethane, 40 ml.; (4) glacial acetic acid, 6 ml. Warm (2) and (3) to 70° C. on a water bath, and add (1) with shaking. Cool, add (4) slowly, and filter. The milk film is stained for half a minute, drained until dry, and washed in water. This is a combined fixing, defatting and staining reagent. Treatment with methyl alcohol for one minute before staining is advisable.

Staining of Fungi.

Fungi are often better examined without preliminary staining. Dilute methylene blue stains most fungal tissues. Dead yeast cells can in general be distinguished from living cells, since only the dead cells take the stain when applied in highly diluted form (twenty minutes' exposure of yeast suspension to 0·1 per cent. aqueous methylene blue). Much, however, depends on the pH of the yeast suspension.

In examining industrial products, such as textiles, it is often desirable to stain fungal hyphæ present in contrast to the material in which they are to be detected.

An excellent mounting and staining medium for this and almost all other cases in which fungi are to be examined is as follows:

Lactophenol	lactic acid	20·00 grms.
	phenol ..	20·00 „
	glycerol ..	40·00 „
	water ..	20·00 „
Cotton Blue		0·05 grm.

The cotton blue is taken up by fungal hyphæ and spores, and the lactophenol forms a mounting medium of suitable refractive index. Where more intense staining is desired, the material may be first warmed in 2 per cent. aqueous cotton blue, washed in water, further decolorised by warming in lactophenol, and finally mounted in lactophenol.

It may be mentioned that the standardisation of cotton blue by stain manufacturers is imperfect, and some kinds give better results than others. Aniline blue (B.D.H.) is very satisfactory.

Aqueous picro-aniline blue is also a useful stain for fungi. For more complicated fixing and staining methods for botanical sections, reference may be made to Rawlins (1933). Other useful textbooks are given in the list below.

REFERENCES

CHAMBERLAIN, C. J. (1932): Methods in Plant Histology. Chicago.

CLARK, W. M. (1928): The Determination of Hydrogen Ions. London.

DAVIS, J. G., AND ROGERS, H. J. (1938): Soc. Agric. Bacteriologists. Abst. of Proceedings.

EDWARDS, S. J. (1933): J. Compar. Path., **46**, 211.

EYRE, J. W. H. (1930): Bacteriological Technique. London.

FRED, E. B., AND WAKSMAN, S. A. (1928): Laboratory Manual of General Microbiology. New York.

GARNER, W. (1932): Industrial Microscopy. London.

MACKIE, T. J., AND MCCARTNEY, J. E. (1931): Practical Bacteriology. Edinburgh.

MUIR, R., AND RITCHIE, J. (1937): Manual of Bacteriology. London.

RAWLINS, T. E. (1933): Phytopathological and Botanical Research Methods. London.

SALLE, A. J. (1939): Fundamental Principles of Bacteriology. New York.

WILSON G. S., *et al.* (1935): Med. Res. Council. Special Report Series—No. 206, The Bacteriological Grading of Milk. H.M. Stationery Office.

CHAPTER VII

METABOLISM OF MICRO-ORGANISMS

Structure and Composition.

THE general term *metabolism* is used for all the processes brought about by the vital activities of an organism. It includes the utilisation of food substances, the production of energy for growth and multiplication, and the formation and excretion of waste products.

The cells of micro-organisms are bounded by cell walls, whose composition, though still of an uncertain nature, is less closely related to cellulose than are the cell walls of higher plants. The existence of this membrane implies that food can only be absorbed in solution. No solid food can be utilised unless it is first converted into a soluble form; this may occur through the action of an enzyme produced by the micro-organism—*e.g.*, starch is liquefied by the enzyme amylase (diastase). The old classification of enzymes into endo- and exo-enzymes, according to their power of diffusion outside the cell wall, is not a very sharp one. Both amylase and cellulase, which liquefy the insoluble substances starch and cellulose respectively, presumably act outside the cell; yet whereas the former usually diffuses out readily into a liquid medium, the latter has never been isolated from living fungi or bacteria. Some sort of linkage with the living organism seems to be essential in the case of cellulase.

Within the cell wall of fungi is the colloidal complex, *protoplasm*, about whose structure and chemical composition we still know very little. Included in the protoplasm are *nuclei* similar to those of higher plants, but not always restricted to one per cell, aqueous vesicles known as *vacuoles*, and reserve food products such as fat, glycogen or volutin. The structure of bacteria is less easily seen owing to their small size, and it is uncertain whether true nuclei exist in this group.

Growth and Energy Requirements.

For growth and multiplication a suitable food supply is necessary, and most micro-organisms require to have carbon and nitrogen supplied in an organic form. In this they resemble animals and differ from green plants, which can make use of carbon dioxide from the atmosphere and nitrates from the soil. Exceptions as regards nitrogen are many fungi and yeasts, and some bacteria, which can utilise nitrates or ammonium salts; also certain forms of bacteria which are capable of " fixing " atmospheric nitrogen.

In addition to hydrogen, oxygen, carbon and nitrogen, micro-organisms require smaller amounts of potassium, phosphorus and sulphur, together with traces of other elements—notably calcium, magnesium and iron—whose essential nature has been demonstrated only in the case of special organisms.

Micro-organisms require food both for building up their cell substance and for the production of energy. The energy is necessary for the transformation of food into cell substance and for other vital processes—*e.g.*, motility.

The *autotrophic* bacteria obtain their energy by the oxidation of inorganic substances, and can utilise atmospheric carbon dioxide as a source of carbon. These special types (among which are the nitrifying bacteria) require no organic nutrient at all. Other organisms—*e.g.*, urea decomposing bacteria—get their energy from the hydrolytic decomposition of urea and related substances, but require carbon-containing compounds for cell building. Many soil organisms can utilise phenolic compounds as their source of energy. *Heterotrophic* micro-organisms, which constitute the majority, obtain both energy and carbon from organic compounds, such as carbohydrates, proteins, etc., either by oxidation or by effecting intramolecular structural changes. For an account of bacterial nutrition see Knight (1936).

Most micro-organisms, like animals, bring about the oxidation of organic matter by a process of respiration, atmospheric oxygen being replaced by carbon dioxide in the process. Micro-organisms that require free access of oxygen are termed *aerobic*. Many bacteria and most fungi are of this type. Other organisms fail to grow if too much oxygen is present and are termed *anaerobes*. The so-called strict anaerobes—*e.g.*, the bacterium

of tetanus and the butyric acid bacteria—cannot tolerate any free oxygen at all. The majority of bacteria and yeasts, however, are *facultative anaerobes—i.e.*, they are capable of growth under both conditions, but in the anaerobic case the required energy is obtained from internal changes in suitable organic matter and not from simple oxidation. During this process many side products of incomplete oxidation are formed—*e.g.*, alcohols, organic acids, etc.—and it is the formation of such substances—frequently accompanied by the evolution of gases—which is the basis on which the fermentation industries are founded.

According to Waksman (1932), while fungi transform up to 50 per cent. of food substrate into mycelium, aerobic bacteria transform a lower percentage into synthesised cells, and anaerobic bacteria something of the order of 1 per cent. Hence for equal amounts of growth the anaerobic bacteria give rise to much greater initial decomposition of organic matter.

The decomposing activities of micro-organisms are governed largely by the carbon-nitrogen ratio of the organic food material. Carbohydrates are much better sources of energy and carbon than are proteins, and when the former are present in excess the decomposition of proteins takes effect only in so far as the needs for nitrogen are met. When the carbohydrate content is low, proteins are broken down to make up for this deficiency. The maintenance of a correct carbon-nitrogen ratio is therefore frequently of economic importance, particularly in relation to questions of soil fertility.

Environmental Factors.

The absorption of food is an osmotic process, and if the fluid medium in contact with the organism is too concentrated, shrinkage or *plasmolysis* of the cell contents may occur. There is a great variation in the osmotic concentrations that different organisms will tolerate. Most bacteria cannot endure 10 per cent. salt solution, yet certain salt-tolerant types will grow in a 25 per cent. solution. Some—the obligate halophiles—actually require a high salt concentration for normal growth. Certain moulds and yeasts are capable of growth in the presence of 60 to 70 per cent. sugar. Tolerance to high concentrations

of dissolved substances is to some extent bound up with the question of the minimum moisture requirement of the organisms. Mould fungi can exist in a drier substratum than can bacteria or most yeasts. In the case of an organism exposed to the atmosphere it has been shown that the governing factor —assuming that equilibrium has been reached—is not the moisture content of the substratum, but the relative humidity of the atmosphere.

The *temperature* requirements of micro-organisms also show wide variation. Certain fungi can grow even at 0° C. or less, and some of these develop on goods in cold storage. For the majority of fungi the optimum temperature is in the region of 25° C., though for a few moulds it approaches 40° C. Bacteria do not develop appreciably at freezing point, and their optimum temperature for growth is usually 25°-40° C. The group known as *thermophilic* bacteria are capable of healthy growth at the surprisingly high temperature of 65° C.

Light is not essential to the growth of most fungi and bacteria, since they contain no chlorophyll. The absence or presence of light may affect certain growth characters, and bright sunlight has a germicidal effect. Most micro-organisms, however, are not greatly affected by light of normal intensities.

The importance of the *reaction* of a medium—its acidity or alkalinity—was long ago recognised by Pasteur. The introduction of the pH scale has brought greater precision in recording such effects, and the optimum hydrogen-ion concentration for many organisms is now known. At times an exaggerated importance has been given to exact pH control. In general, however, micro-organisms have a certain tolerance or power of adjustment. Bacteria usually grow best at a neutral or slightly alkaline reaction, whilst fungi prefer an acid medium.

Influence of other Organisms.—The growth of an organism is sometimes stimulated or inhibited by the presence of another species.

Enzymes.

The small size and consequent large ratio of surface area to volume possessed by micro-organisms makes them capable of carrying out the chemical conversion of a large mass of material in an extraordinarily short time. It was once

customary to try to represent any such action by a suitable equation, the best known example being the equation for the alcoholic fermentation of dextrose by yeast: $C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$. Such a process is now regarded as a complex of simultaneous reactions, the end products of which can be varied to a considerable extent by variations in temperature, pH control, aeration, and composition of the medium. The alcoholic fermentation referred to, for example, may be modified in several ways so as to give a high yield of glycerol. In the fermentation of dextrose by mould fungi, yields of citric acid have been obtained far in excess of " theory " according to the older views on the chemistry of this process.

Similarly the endless multiplication of *enzymes* by postulating a fresh enzyme to account for each new enzymatic reaction met with is now looked on with disfavour. Before considering the alternative view, it would be as well to consider the nature of enzymes a little more fully.

In the early days of microbiology, the organisms responsible for the various processes of fermentation, putrefaction, and decay were called " ferments." Later on it was found that a non-living substance capable of carrying out the reaction could in certain instances—*e.g.*, Buchner's " zymase " from yeast—be extracted from the organism. These " unorganised ferments," as they were called to distinguish them from the " organised ferments " or living organisms, are now termed *enzymes*. Similar enzymes are present in green plants and in animals. The enzyme hydrolysing starch, for example, is found in bacteria, in fungi, in higher plants, and in animals, and it is a very interesting fact that preparations from all four sources are used industrially for the removal of starch.

An enzyme may be defined as an organic catalyst produced from a living cell, but its exact nature is still a matter of controversy. Some enzymes are said to have been isolated in the crystalline state and shown to be proteins. The usual modern view is that an enzyme consists essentially of a chemically active group attached to a colloidal carrier. It resembles an inorganic catalyst in that it alters the rate of a reaction without changing the final equilibrium, and without entering into the end products. In certain cases—*e.g.*, maltase—it has been found possible to demonstrate the reversibility of enzyme

action. In the living cell probably all enzyme actions are reversible.

Enzymes, like the living cells in which they were formed, may be destroyed by heat or antiseptics, and are only active within a definite range of hydrogen-ion concentration. They are, however, more resistant than micro-organisms. Their optimum temperature usually lies between 40° and 60° C.; they are not destroyed by alcohol; and some of them can act in the presence of toluol, thymol, and certain other antiseptics that check growth of bacteria and fungi.

The rate of reaction is in general proportional to the concentration of enzyme present.

Most enzyme preparations are largely in the nature of chemical curiosities, and it is far more economical to use the living micro-organisms wherever possible. Most of the work on the theory of alcoholic fermentation (Harden, 1932-33; Meyerhof, 1933) has been carried out with *zymase* or yeast enzyme preparation, but no distiller or brewer has yet been tempted to abandon the use of yeast for the feeble action obtained by this means.

Amylase (diastase) preparations are the most active and widely used, but even these cannot act on raw starch as can the living organisms. Certain other enzyme preparations capable of industrial use will be referred to in subsequent chapters.

Chemistry of Metabolism.—The modern tendency is to regard biological metabolism in terms of oxidations and reductions, and theories of fermentation on this basis have been elaborated, more especially in the case of the carbohydrates.

The most comprehensive scheme is that of Kluyver and others, which incorporates much of the previous work of Wieland, Warburg, and Neuberg. Enzymes or other biochemical catalysts, according to Kluyver (1931), act on the substrate and its decomposition products, causing certain hydrogen atoms to become " activated " so that they can either combine with an available " hydrogen acceptor "—often atmospheric oxygen—or become stabilised as molecular hydrogen which is then evolved as a gas. Oxygen activation, according to Warburg's views, also occurs, the catalytic agent being the iron contained in living cells.

Within the last few years such theories have been reviewed

and extended in terms of oxidation-reduction potentials—*i.e.*, oxidations and reductions have been regarded in terms of the corresponding loss or gain of electrons; the state of equilibrium of any system can thus be measured by the potential of an electrode or by suitable colour indicators as is done in the case of hydrogen-ion concentration.

The extensive research devoted to the yeast fermentation of dextrose, mostly based on work with zymase preparations and not with the actual yeast organism, has brought out the interesting fact that the first step is the formation of esters with phosphoric acid. The esterified dextrose molecule then breaks up into triose phosphate, and two molecules of the latter react together to yield α-phosphoglyceric acid and α-glycerophosphoric acid. From these two substances there are formed, by further oxido-reductions, other products which under normal fermentation conditions ultimately undergo an almost complete conversion into ethyl alcohol and carbon dioxide, in proportions represented by the equation quoted above. Traces of other substances—aldehydes, ketones, esters, organic acids, and higher alcohols—are also present, and arise by complicated side reactions, showing that the processes involved are by no means as simple as the equation suggests.

Kluyver (1931) has endeavoured to bring out the essential similarities of all the known carbohydrate fermentations by yeasts, moulds, and bacteria, and has succeeded in reducing such processes to a comparatively small number of reactions combined in various ways. Future work will probably bring a corresponding simplification in our knowledge of protein fermentations.

Recent advances in the biochemistry of moulds and bacteria are discussed by Clutterbuck (1936) and Walker (1936) respectively.

Decomposition of Carbohydrates.

Carbohydrates are not essential to the growth of all micro-organisms. Many bacteria and fungi can obtain the necessary carbon by decomposition of proteins, fats, or in some cases from alcohols or organic acids. In view, however, of the important part played by starch, cellulose, and sugars in the

constitution of plant products, the decomposition of carbohydrates has received most attention from microbiologists.

Sugars.—A great deal of work has been carried out in order to find out what happens when a simple sugar is broken down by micro-organisms. It appears probable that a hexose first forms phosphoric esters, then breaks up into compounds with a smaller number of carbon atoms, and finally undergoes a series of hydrogen transfers whose exact nature depends both on the organism and on the conditions of its environment. The final products of complete oxidation of sugars are water and carbon dioxide, the former being used by the micro-organism for growth and the latter given off as a gas. The decomposition process, however, is usually incomplete (*i.e.*, the carbon present is not all oxidised to CO_2), and may be predominantly aerobic or anaerobic. The former type gives rise to acids such as gluconic, fumaric, oxalic, and citric; the latter to alcohols and acetone, and to lactic, acetic, butyric, and propionic acids (see Chapter X.). Some of the more important decompositions are as follows:

(1) Hydrolysis of disaccharides to monosaccharides—*e.g.*, maltose to 2 molecules of dextrose; saccharose to dextrose+levulose; and lactose to dextrose+galactose.

(2) Complete oxidation to carbon dioxide and water is produced by yeasts and moulds acting under aerobic conditions.

(3) Alcoholic fermentation takes place when yeasts and certain moulds act on sugar under anaerobic conditions. The chemistry of this process has already been discussed briefly.

(4) Lactic acid production by the true lactic bacteria, capable of occurring under anaerobic conditions.

(5) The *coli-aerogenes* type of fermentation, also facultatively anaerobic, and producing both acid and gas, the latter being a mixture of hydrogen and carbon dioxide.

(6) Butyric acid formation under strictly anaerobic conditions.

Fungi and bacteria show considerable specificity in the sugars they can utilise, and in systematic bacteriology the

ability to ferment various sugars is used as a diagnostic feature.

Pentoses are less commonly attacked than hexoses; yeasts are unable to ferment them, although they can utilise them to some extent. Some mould fungi and bacteria use pentoses readily. Free pentoses, however, are rare in nature, being locked up as pentosans, and the difficulty in the preliminary hydrolysis of these widely distributed substances restricts their economic utilisation.

Disaccharide sugars may or may not be available to an organism, according to its power of hydrolysing them into simpler form. Saccharose, or cane sugar, is available to most yeasts and fungi, and to many bacteria. Maltose and lactose are also attacked by many organisms.

Glucoside hydrolysis has been studied principally in relation to indican (whose breakdown in the natural indigo fermentation is principally due to bacteria), and tannin.

Starch.—Starch is produced by most plants as a reserve food material. Cereal grains usually contain 60 to 80 per cent. starch, originally destined to feed the young plant on germination, but frequently diverted by man to his own use.

Many bacteria, and most fungi and actinomycetes, secrete the enzyme amylase (or diastase), which converts starch into soluble products—dextrins, maltose, and finally dextrose—that can pass through the cell wall and be utilised by the organism. Living organisms can carry out this hydrolysis of starch on the raw material, whereas non-living enzyme preparations of amylase act slowly or not at all unless the starch first made into a paste with hot water, causing swelling and bursting of the starch granules.

The sugars produced by starch hydrolysis are utilised as already described—as sources of energy obtained by their oxidation to carbon dioxide and water, or more usually an incomplete oxidation to organic acids, alcohols, etc., with the evolution of carbon dioxide and possibly also of hydrogen or other gases.

Cellulose.—Cellulose, as the skeleton of all plant tissues, is a product whose abundance need not be stressed. It is much more resistant to attack by micro-organisms than is starch. Many of the common mould fungi—*e.g.*, *Aspergillus fumigatus*—decompose cellulose. The higher fungi also include many

forms active in this respect—the bracket fungi that attack timber trees, and dry-rot and similar rots of structural timber. Bacteria decomposing cellulose may be aerobic—*e.g.*, *Cytophaga hutchinsoni*—or anaerobic; the latter type includes certain thermophilic bacteria producing hydrogen or methane as gaseous products of decomposition.

The stages in the enzymatic breakdown of cellulose are probably cellulose—cellobiose—dextrose, the dextrose becoming available for further decomposition within the organism.

Hemicelluloses.—Lignocellulose is not attacked readily by most organisms, but certain of the timber-destroying fungi—*e.g.*, *Polyporus annosus* and *Trametes pini*—attack the lignified tissue, the resulting conditions being termed " white rots."

Pentosans and *hexosans* are widely distributed in plants, but are resistant to attack by most organisms. Their breakdown in nature is probably due principally to the slow action of certain mould fungi present in the soil.

Decomposition of Fats.

Certain micro-organisms are capable of decomposing fats, splitting them into glycerol and free fatty acids. Both these products are available for further decomposition, the glycerol being readily utilised by the organisms, and the acids oxidised more slowly.

The enzymes concerned in fat decomposition are known as *lipases*, and the result of their action is the development of rancidity. Lipolytic action is of economic importance in connection with the food and textile industries, and will be again referred to under those heads.

Decomposition of Proteins.

From the point of view of nutrition, proteins are important as a source of organic nitrogen. They are compounds of carbon, hydrogen, oxygen and nitrogen; in some cases sulphur and phosphorus are also present. Breakdown through the action of acids, enzymes, or micro-organisms passes through the stages protein — proteoses — peptones — amino-acids. Further decomposition by micro-organisms gives rise to ammonia and carbon dioxide, or to intermediate products

such as amines. When decomposition takes place under anaerobic or semi-anaerobic conditions the products include many evil-smelling compounds (hydrogen sulphide, tri-methyl-amine, indol, skatol, etc.), and the process is popularly known as putrefaction.

The soluble proteins such as gelatin and albumen are fairly readily decomposed, but the more resistant forms typified by hair, horns, feathers are attacked only by a limited number of organisms.

Protein decomposition has been less completely studied than carbohydrate decomposition. Both are of great economic importance in connection with spoilage problems and sewage disposal. Carbohydrate breakdown processes, however, in view of their economic utilisation and the greater simplicity of the chemical reactions involved, have received more attention from research workers.

Decomposition of Inorganic Substances.

These processes are either oxidations or reductions. Among the former, which yield energy and are effected mainly by autotrophic bacteria, the oxidation of ammonium salts to nitrates by the *Nitrosomonas-Nitrobacter* groups is of great importance in maintaining the fertility of the soil. Other oxidations bring about the conversion of sulphites and sulphides to sulphates and ferrous to ferric compounds. In the reducing reactions, the associated micro-organisms require organic matter as food, but obtain the oxygen necessary for its decomposition by the reduction of inorganic substances. Thus nitrates are reduced to gaseous nitrogen, an undesirable reaction in that it decreases the value of plant manure; sulphates are reduced to sulphur and sulphides; and arsenious compounds to arsine. The last reaction will be referred to again in Chapter XIV.

Synthetic Processes.

Some of the synthetic activities of micro-organisms have economic significance. A process of which some use has been made under the pressure of war-time conditions enables atmospheric nitrogen to be converted into a protein food

material within two or three days. First the nitrogen is converted into ammonia by the Haber process; ammonium salts are then used as a nitrogen source for the growth of yeast in a solution of molasses, and thus the nitrogen is converted into organic form.

Certain yeasts (more particularly *Endomyces* sp.), and strains of *Oospora lactis*, are very rich in fats, and offer a means of converting sugars into fats.

One of the most interesting synthetic activities of micro-organisms is the production of polysaccharides. In addition to glycogen, which is a reserve product of the yeast cell, and starch or a starch-like substance formed by certain bacteria and fungi, mucous substances are produced. Viscous substances in crude juices of beet and cane sugar have long been known as a cause of manufacturing difficulties, and are now known to be due to capsule formation by such organisms as *Leuconostoc mesenterioides*. Mucus is also produced by other bacteria common in flour, milk, etc., and by certain fungi. The substance produced by *L. mesenterioides* is known to be a condensation product of dextrose, although it is formed more readily from saccharose than from dextrose or from invert sugar. Under suitable conditions 50 parts of gum may be obtained from 100 parts of cane sugar within a few days. Other such substances are known, some being condensation products of levulose, and in many cases probably contain combined nitrogen.

Diplococcus pneumoniae and certain other pathogens also form capsules of this nature, some of which are toxic or are capable of producing antibodies, and are therefore of great interest in the study of immunity.

Other Products of Metabolism.

Heat.—The decomposition of organic substances such as carbohydrates, proteins, and fats liberates energy which is available for growth. The micro-organisms responsible for the breakdown, however, frequently fail to utilise all the energy liberated, and the balance appears in the form of heat. The heating up of manure heaps, silos, or haystacks is well known.

Light.—The phosphorescence of decaying wood sometimes observed is due to certain fungi, notably to *Armillaria mellea*.

Even more marked is the light produced by certain bacteria; such types can be isolated—*e.g.*, from phosphorescent meat or fish—on laboratory media of suitable composition, and the light production studied. The cultures must usually be looked at for some minutes in a dark room before the luminosity is apparent, and it will be found that if the oxygen supply is cut off, light production ceases immediately, showing that it is an oxidation process.

Odours.—Apart from the characteristic odours of recognisable products of metabolism, such as butyric acid or ammonia, many micro-organisms produce traces of aromatic substances whose composition is uncertain. Yeast fermentations give a fruity smell due to mixed esters, actinomycetes have a characteristic nutty or earthy flavour, and mould fungi usually cause a musty smell suggestive of phenolic compounds.

Pigments.—Coloured substances may be produced within the cell, as in the dark-coloured mycelium of *Cladosporium* and in the yellow chromogenic bacteria and pink yeasts. Or the pigment may be produced in the medium, as in the red and purple pigments produced by species of *Penicillium* and *Fusarium* in starch media, and the green pigment of *Pseudomonas aeruginosa* in broth. Little is known as to the chemical constitution of such pigments, and attempts to make economic use of them have so far met with no success.

Toxins.—These are complex chemical substances of unknown composition, small traces of which seriously interfere with animal metabolism and cause disease or death. They are sensitive to heat, the majority being rapidly destroyed at 100° C.

Toxin production is not confined to micro-organisms, but many pathogenic bacteria—*e.g.*, the organisms of tetanus and botulism—produce powerful toxins. The most deadly toxin produced by fungi is that of *Amanita phalloides*, a fungus sometimes mistaken for the common edible mushroom.

REFERENCES

BERNHAUER, K. (1936): Gärungschemischer Praktikum. Berlin.

BUCHANAN, R. E., AND FULMER, E. I. (1928-30): Physiology and Biochemistry of Bacteria. 3 vols. London.

CLUTTERBUCK, P. W. (1936): J. Soc. Chem. Ind., 55, 55T.

HARDEN, A. (1932): Alcoholic Fermentation. London.

—— (1933): J. Inst. Brewing, 39, 644.

HIND, H. L., AND SCHOEN, M. (1928): The Problem of Fermentation. London.
KLUYVER, A. J. (1931): The Chemical Activities of Micro-organisms. London.
KNIGHT, B. C. G. (1936): Medical Research Council, Special Report 210. H.M. Stationery Office.
MARSHALL, C. E. (1921): Microbiology. London.
MEYERHOF, O. (1933): Nature, **132**, 337 and 373.
RAISTRICK, H., *et al.* (1931): Phil. Trans. Roy. Soc., B.220, 1.
STEPHENSON, M. (1930): Bacterial Metabolism. London.
THAYSEN, A. C., AND BUNKER, H. J. (1927): The Microbiology of Celluloses, Hemicelluloses, Pectins, and Gums. London.
—— AND GALLOWAY, L. D. (1930): The Microbiology of Starch and Sugars. London.
VAN LAER, M. H. (1935): Chimie des Fermentations. Paris.
WAKSMAN, S. A. (1932): Principles of Soil Microbiology. London.
—— AND DAVISON, W. C. (1926): Enzymes. London.
WALKER, T. K. (1936): J. Soc. Chem. Ind., **55**, 61T.

CHAPTER VIII

CONTROL OF MICRO-ORGANISMS

BEFORE considering methods of control as applied to any particular instance, it is first desirable to know what organism or organisms are predominantly concerned. This requires careful microscopical and experimental work, combined with the application as far as possible of "Koch's postulates" described in Chapter XIII. Once the fungus, yeast, or bacterium causing trouble is isolated and identified, the investigator is in a much better position to consider how its activities may be controlled.

Methods for the control of micro-organisms may be summarised under six main factors essential for growth. These are: (1) infection, (2) moisture, (3) food supply, (4) suitable temperature, (5) suitable atmosphere, and (6) absence of inhibiting substances, such as antiseptics, acids or alkalies. When control can be effected by modifying two or more of the above factors, the total effect is usually greater than the sum of the individual effects (Tomkins, 1929).

The essentials for growth as classified above will now be considered more fully.

1. **Infection.**—Moulds, yeasts and bacteria, especially in the form of spores, are constantly settling from the atmosphere. Few materials can be kept sterile for long, unless they are sterilised in sealed containers, as in the case of canned foodstuffs. Cleanliness, refrigeration, speed of transit, wrapping, etc., all reduce the amount of infection and minimise the chances of troubles due to micro-organisms. Wrapped bread and certified milk represent an approach to this ideal of asepsis.

To reduce atmospheric infection a still atmosphere is desirable. In certain cases it may be worth while to fit a room with air filters; one such type of filter consists of a frame filled loosely with metal fragments covered with a layer of viscous oil that traps the air-borne particles. Walls with a smooth

finish, the rounding of all " corners," and the absence of ledges that cannot readily be cleaned, are a help in reducing dustiness. Perishable material should not be left lying about, and work benches, floors, etc., should be well washed with soap and hot water or with ½ per cent. hypochlorite solution. Machinery, especially food plant, may require careful microbiological examination (of swabs, etc.), as also may the water supply and the various ingredients used in the process under consideration.

2. **Moisture.**—No development of micro-organisms is possible without moisture. Bacteria and yeasts are more exacting in this respect than are certain fungi. In the case of dry materials in equilibrium with their storage atmosphere, the moistness of this atmosphere is the important factor, and it is better to think in terms of relative humidity than of moisture content or moisture regain (Tomkins, 1929). Some mould fungi—*e.g.*, the *Aspergillus glaucus*, *A. candidus* and *A. versicolor* groups—are capable of slow growth even at 75 per cent. relative humidity, whereas the majority of fungi require 85 to 95 per cent. (Galloway, 1935). Yeasts, bacteria, and fungi parasitic on plants need moisture approaching or exceeding saturation point. Thus the best method of preventing the deterioration of " dry " products is to keep the storage atmosphere down to 70 per cent. relative humidity. Should it exceed this, it may be brought down to the required figure by raising the temperature a few degrees, through the installation of a steam pipe or some such method. We thus have the paradox, often puzzling to the uninitiated, that a closer approach to the optimum temperature for growth of an organism may be the best way of preventing the growth of that organism altogether.

Sudden drops in temperature leading to condensation are to be avoided. In the case of " damp " products not in equilibrium with their storage atmosphere, the latter should be kept as dry as is permissible.

In the majority of cases of microbiological damage, control of the moisture conditions offers the cheapest and most effective means of suppressing the undesirable organisms. The organisms are not necessarily killed, for many bacterial and fungal spores can survive for long periods in a dry condition, but growth is prevented. The preservation of foodstuffs by drying is dealt with in Chapter IX.

The sling hygrometer offers a convenient instrument for the measurement of relative humidity. For laboratory experiments on the effect of atmospheric humidity, the requisite relative humidities can be obtained by means of suitable solutions—either saturated solutions of salts, or solutions of sulphuric acid or calcium chloride made up to a certain specific gravity—50 ml. of solution being placed in a stoppered jar not exceeding 500 to 750 ml. capacity and incubated at constant temperature. The material to be tested is suspended in the air space over the liquid. Thus a saturated solution of potassium chloride at 25° C. gives 85 per cent. relative humidity, which may also be obtained over a calcium chloride solution of specific gravity 1·15 (measured at 15° C.). For data on saturated solutions reference may be made to Intern. Crit. Tables (1926-33), and for sulphuric acid solutions to Wilson (1921).

Under the heading of moisture requirements may be considered the osmotic effects of salt and sugar solutions. The cells of micro-organisms are bounded by semi-permeable membranes, and if placed in very strong solutions the cells become *plasmolysed*, the protoplasm shrinking away from the cell wall. Bacteria differ widely in their reaction to solutions of common salt, and the selective action of salt is the basis of the methods for pickling preservation of salted pork, etc., and salting of butter.

Strong sugar solutions are employed for the preservation of fruits and condensed milk. The freedom of jams from surface moulds depends mainly on the concentration of sugars present. A few yeasts and moulds are extraordinarily tolerant of strong sugar solutions and can develop even in the presence of 65 per cent. cane sugar; it is not unexpected that the moulds developing under such conditions are those—*e.g.*, the *Aspergillus glaucus* group—which are known to have the lowest moisture requirements.

The additional moisture produced by the presence of deliquescent substances does not appear to be generally available to micro-organisms (Galloway, 1935), although certain deliquescents such as glycerol may lead to increased growth of micro-organisms by virtue not of the water they take up but of their nutritive value.

3. **Food Supply.**—In addition to water, a small supply of

some source of carbon and nitrogen, and still smaller traces of certain other elements, are necessary for the growth of micro-organisms. It is possible in some instances to limit growth of infections by cutting off their food supply, but it must be remembered that minute traces will suffice to support quite considerable growth.

Nevertheless, the principle may often be applied in practice. In the textile industries, mildew troubles are often brought about by the use of sizing materials too rich in nitrogenous matter. Soiled eggs are more likely to develop mould growth than are clean eggs, on which the fungi fail to establish themselves. Packing paper, cardboard, or wooden boxes have all been known to contribute to outbreaks of mould growth, and bottles or other vessels which have contained nutrient liquids must be well rinsed and dried after cleaning, or fresh bacteria will develop in the diluted nutrient. Glycerol, added as a softener or for other purposes, provides a readily available food for micro-organisms; similarly a nitrogenous flour paste should if possible be replaced by a paste of pure starch or dextrin.

4. **Atmosphere.**—As described in Chapter VII., some organisms demand aerobic and some anaerobic conditions, whilst others can develop in either. Mould fungi, being strongly aerobic, may be suppressed by the exclusion of air, as in the wrapping of butter and cheese. Conversely, some aeration is necessary in the making of silage and in the fermentation of flour for textile sizing, in order to check the development of butyric bacteria.

The governing factor in the above cases is the presence or absence of oxygen. Moulds, and to a lesser extent bacteria, are susceptible to high concentrations of carbon dioxide in the atmosphere. This is a definite toxic effect and does not simply depend on the reduction of the oxygen supply; if an inert gas or a vacuum is substituted, it will be found that exclusion of oxygen has to be very complete before mould fungi are completely checked. The concentration of carbon dioxide to suppress moulds is about 50 per cent., but in the case of foodstuffs a lower figure of 10 to 20 per cent. usually has to be taken on account of the deleterious effect of higher concentrations of carbon dioxide on certain materials. The modern application of this method to the transport of chilled

meat is dealt with in Chapter IX., but the principle was recorded many years ago (Chapin, 1902) and possible applications to storage of fruit, textiles, etc., have been suggested at various times since.

Recent work by Tomkins (1932) has called attention to the existence of far more powerful volatile antiseptics. It has long been known to laboratory workers that naphthalene vapour used to repel mites (see p. 48) exerts some inhibiting action on the growth of micro-organisms. Thymol is still more active in this respect. Tomkins showed that very minute traces of acetaldehyde or of ammonia in the storage atmosphere completely inhibit fungal growth. So far attempts to apply this discovery to fruit storage methods have not been very successful, but the possibilities are by no means exhausted. Periodic "gassings" with sulphur dioxide, formaldehyde, or nitrogen trichloride (Klotz, 1936) are sometimes possible as a means of checking the development of micro-organisms.

5. **Temperature.**—Sterilisation by heat has been mentioned under the heading of Infection, and also in Chapter IV. A temperature above the maximum for growth of organisms likely to occur, or below the minimum, affords control, but in practice the former is seldom practicable. A temperature of 50° C. inhibits growth of most organisms, but certain thermophilic bacteria still develop well at 65° C. The use of low temperatures is much more generally applicable, and cold storage has become a standard method for preserving foodstuffs and many other perishable articles. A temperature of 0° C. checks yeasts and most bacteria, but by no means all fungi; the development of mould fungi on meat in cold storage is dealt with in Chapter IX.

Even within the temperature range for growth of organisms, temperature exerts an influence on the nature of the type whose growth predominates. Milk or flour paste kept at 20° C. will develop a different microflora from that developing in the same medium at 40° C. Even at temperatures unsuitable for the growth of the organism, enzyme action may still continue.

6. **Inhibiting Substances**—(*a*) *Hydrogen-ion Concentration.*—All micro-organisms have a limited range of reaction at which they can develop, and if the hydrogen-ion concentration of the medium is outside this range growth is checked. Pickles,

silage and other lactic acid fermentations apply this principle, the acidity preventing the development of undesirable bacteria. Less frequently it is practicable to preserve a medium by maintaining an alkaline reaction. The effect of ammonia as a volatile antiseptic has already been discussed, and the mechanism is probably the inhibition of enzyme action at an alkaline reaction.

(*b*) *Antiseptics.*—An antiseptic, or preservative, inhibits growth, whilst a disinfectant actually kills the organism. There is really no dividing line—all inhibiting substances have some killing power—but the distinction is useful in practice, since the ratio of inhibiting power to killing power varies widely for different substances. In giving the toxic concentration of a substance it is important to state the organisms tested, whether antiseptic or disinfectant power is referred to, and if the latter the time required should be stated.

The majority of antiseptics (the term will frequently be used in this chapter to cover disinfectants as well) are poisonous to higher organisms as well as to micro-organisms. This limits their use very considerably, and has led to strict regulations regarding their presence in foodstuffs, tobacco, etc. Even for general purposes the use of a powerful poison like mercuric chloride is frequently undesirable on account of the risk to operatives; yet mercury compounds are now used widely for dusting seed grain (to suppress fungal pests) without harmful results. Fungal antiseptics applied to growing plants as sprays, etc., must supply the happy mean between suppressing the fungus and damaging the plant.

It seems almost unnecessary to point out, though the fact is sometimes overlooked, that it is the *concentration* and not the *amount* of antiseptic that matters. In a mixed medium this concentration should be calculated on the aqueous phase.

Of the common antiseptics, the best known are coal tar products—*e.g.*, cresylic acids. This is possibly the origin of the popular belief that a good antiseptic must have a pronounced odour. That this is not true is shown by mercuric chloride, which although without odour is a powerful antiseptic; organic mercury compounds are also very active, especially against fungi.

It does not always follow that a good bactericide is necessarily a good fungicide, and *vice versa*, though the two properties

usually run parallel. Of the cheaper and more readily available substances toxic to bacteria, the most efficacious are mercury compounds, and in particular mercuric chloride; chlorine, often most conveniently supplied in the form of hypochlorite solutions; chlorinated phenols; formaldehyde, obtainable as a 40 per cent. solution under the name of formalin; sodium silicofluoride, or magnesium silicofluoride if a more soluble form is required, and cresylic acids, " lysol " or other emulsified coal tar products. Gibson (1936) gives a comparative summary of the commoner antiseptics. Mercuric chloride and hypochlorites may lose their efficacy in the presence of organic matter. The latter are apt to lead to corrosion of metal surfaces and to deteriorate on storage, but have particular advantages for certain purposes—*e.g.*, swimming baths, and sterilisation of plant in food factories. Borax, if present in a concentration of 1 per cent., is sometimes a convenient antiseptic. Hydrogen peroxide and iodine are useful for skin disinfection and wounds, and dyestuffs, such as acriflavine, have also been used for this purpose. Further information regarding bactericides is given by Rideal (1921).

Fungi, and especially many of the common mould fungi, are frequently extraordinarily resistant to antiseptics. Some of the most active and economical fungicides may be mentioned here.

Mercury compounds, especially the organic mercury compounds; chlorinated phenols, which suffer from the disadvantage for some purposes that their smell, though faint, is extraordinarily persistent; β-naphthol, often used for glue, and in tanning practice; chlorine (hypochlorites) and formaldehyde (formalin), both of which are volatile and very efficient antiseptics; and many other substances, such as copper sulphate, which are less toxic but often convenient in practice. Salicylanilide was originally recommended for textile purposes, but is finding many other applications. Ortho-phenyl-phenol has a faint persistent smell, but is effective against both fungi and bacteria. Para-chlor-*m*-cresol and *p*-chlor-*m*-xylenol are now available cheaply; the latter is particularly effective against mould fungi. Malachite green is a powerful dyestuff, which in concentrations of 1 : 20,000 or less will suppress most fungi, and this chemical is likely to find an increasing number of applications especially for agricultural and medical purposes; in its less pure form it can be obtained quite cheaply. Para-

nitrophenol is cheap and efficacious, but in neutral or alkaline solution it acts as a yellow dye. Thallium carbonate is a most powerful fungicide, but its use is limited by its very poisonous nature and its prohibitive price. For the toxic concentrations of many of the above fungicides reference may be made to Fargher, Galloway, and Probert (1930) and Burgess (1934). The exact amount required to suppress growth in any medium, however, can only be found out by experiment.

Antiseptics commonly used in laboratory practice are thymol and toluol. Both of these check growth of fungi and bacteria, but permit the action of some enzymes to continue. When toluol is added to a liquid to keep it sterile, it is essential to shake the liquid daily, adding fresh toluol when required, *and checking the sterility by microscopic examination.* Most other antiseptics — *e.g.*, mercuric chloride and formalin — inhibit enzyme action as well as the growth of micro-organisms. Copper, in traces of 0·5 part per million or less, suppresses algæ, protozoa, and many bacteria. Zinc salts are cheap and fairly efficient as antiseptics, and nickel salts also exert an antiseptic action. Silver and gold in the form of colloidal solutions are effective bactericides for specific cases. The oligodynamic action of metals on bacteria serves as a basis of many patented processes for the sterilisation of milk, water, etc., but the application of heat is usually cheaper and more efficacious. Benzoic, boric and salicylic acids are all weak antiseptics, formerly used in the food industries, but their use is now restricted or prohibited for this purpose. Sulphurous acid is still used for meat products, fruit juices, etc., in permitted concentrations.

A peculiar effect that is sometimes observed is that an antiseptic in low concentration may not only fail to check growth, but may even exert a marked stimulating action on growth of micro-organisms. This effect would appear to be more marked with mould fungi than with bacteria.

Chemical Constitution and Antiseptic Value.—In certain cases there appears to be some correlation between these two properties, but evidence obtained from much careful and prolonged research in this direction has failed to lead to any consistent theory. A chemical modification improving the toxicity towards fungi may produce the reverse effect towards bacteria. Micro-organisms themselves show large specific

differences. Thus certain species of *Penicillium* are extraordinarily resistant to mercury compounds, withstanding twenty times the concentration that suppresses most moulds. Similar resistant types may be found to most antiseptics if a sufficiently large number of organisms is tested. Probably most cases of toxic action are linked up with the relative molecular structure of the antiseptic and the protoplasm of the host. Since little is known about either, and especially about the latter, it is not surprising that theories break down.

The Effect of pH on Antiseptic Action.—Many antiseptics are most effective within a definite range of reaction. It is therefore necessary to choose an antiseptic with reference to the actual conditions of acid or alkaline reaction under which it will be used in practice.

The Effect of Oils, Fats and Waxes on Chemical Action.—Little attention has been given in the past to this important factor, which is often the cause of unexpected failures. It was observed long ago, for example, that thymol is much less efficient in milk than in aqueous solutions. Many organic antiseptics are more soluble in fats than in water, and in an emulsion the aqueous phase thus becomes depleted of antiseptic (Galloway, 1934). The effect can be demonstrated not only in fine emulsions, but in systems where the contact of the two phases is quite local—*e.g.*, an agar medium in contact with a film of wax.

Testing of Antiseptics.—It is obvious that no one test will give a complete indication of the efficacy of an antiseptic. The nature of the organism to be suppressed, and the nature of the medium in which it is to be suppressed, will both affect the result. Consequently the only satisfactory test of an antiseptic for any particular purpose is to use it for that purpose over an extended period.

As a general indication of the toxic nature of a substance, the custom has arisen of comparing its killing power with that of pure phenol under standard conditions and for a standard organism. The standard organism generally used is *Eberthella typhosa* (*Bact. typhosum*), the bacterium associated with typhoid fever, grown in a standard broth medium of definite pH; sometimes *Bact. coli* is used, and this has the advantage from the point of view of handling that it is not pathogenic.

It must be emphasised that phenol coefficients are only of

use in comparing phenolic compounds as regards their killing (disinfectant) power. They are useless in comparing inhibiting (antiseptic) values.

In Great Britain and the Colonies the Rideal-Walker method is usually employed. This, briefly, is as follows: 0·2 ml. of a twenty-four-hour broth culture of *E. typhosa* at 37° C., cooled to 17°-18° C., is added to 5 ml. of a suitable dilution of the disinfectant in sterile distilled water and allowed to stand for a specified time in a water bath at 15°-18° C. After standing for intervals of 2½, 5, 7½ and 10 minutes, a 4 mm. loopful of the mixture is transferred to tubes of nutrient broth, and the tubes incubated at 37° C. for growth. The object is to find a dilution of the disinfectant which will kill the test organism in 7½ minutes, but not in 5 minutes. As a 1 per cent. solution of pure phenol in sterile distilled water does this, the ratio of the required dilution of the disinfectant to the $\frac{1}{100}$ dilution of the phenol is the Rideal-Walker coefficient. It is customary to make a series of four dilutions of the disinfectant, and a $\frac{1}{100}$ dilution of phenol. Taking these five tubes in turn, a loopful of inoculum is removed every half-minute, and the process repeated three times to give the above stated exposure periods (see British Standards Specification, 1934).

Since little protective organic matter is present the conditions are more favourable for disinfectant action than those likely to obtain in practice. Hence a disinfectant showing a low lethal property in this test will certainly be unsatisfactory under practical conditions. There are many modifications of the Rideal-Walker test, differing only in matters of detail. In the Chick-Martin test dried human fæces is introduced into the medium (see Med. Res. Council, 1931, vol. 9). This test has now been modified to use a yeast suspension in place of fæces (British Standards Specification, 1938). The Admiralty test (Patterson and Frederick, 1931) uses artificial sea-water. In America the U.S. Hygiene Laboratory and the Foods and Drugs Administration both employ broth; the latter also recommends a filter-paper method which is worth noting here.

Filter paper wetted with a broth culture of *Staphylococcus aureus* is placed in the disinfectant solution for some time, transferred momentarily to broth and then to fresh broth and

incubated for growth. In testing volatile substances the filter-paper culture is allowed to dry before exposure to the fumigant.

All the above " hit or miss " killing methods are really tests on the few resistant individuals of a variable bacterial population (Thaysen, 1938). Some workers prefer to treat equal quantities of a bacterial suspension for stated times with suitable concentrations of disinfectant and then to " plate out " and count the colonies. Here it is important to know that sufficient disinfectant is not carried over to produce an inhibiting effect.

For testing *antiseptic* (inhibiting, not killing) power, the agar plate method may be employed. Plates of a suitable agar medium, containing appropriate strengths of antiseptic, are inoculated with a pure culture of the test organism—bacterium or fungus—and the concentration that just suppresses growth is recorded. The antiseptic may if necessary be added to the agar after sterilisation. For ointments, plasters, adhesive tapes, etc., the material can be placed at the centre of the agar plate, and the width of the clear zone in which no organisms develop may be used as a measure of the efficacy of the antiseptic.

When fungi are used as the test organisms, very full information may be secured by the plate method. The agar is inoculated (with the plate inverted) by placing a few spores or fragment of an agar culture at the centre, and is then incubated. After some days the spores develop into a circular colony, which grows at a regular linear rate until the sides of the petri dish are approached, when there may be a slowing of growth. By this method not only can the inhibiting concentration be determined, but also the reduction in growth rate caused by lower concentrations (Morris, 1926). A further method is described by Tomkins (1937).

Test Organisms.—For the Rideal-Walker test *E. typhosa* is used, as stated; this is an organism very easily killed, unlike, for example, the spores of the anthrax bacillus. For testing wound antiseptics, the pus-producing *Staph. aureus* is often used. The desirability of using a wider range of organisms in any general test has been emphasised by a number of workers. The wide specificity of mould fungi, especially species of *Penicillium*, has been demonstrated by Fargher, Galloway and Probert (1930).

It must be borne in mind that antiseptic tests in laboratory media, though giving valuable information, should always be followed by experiments on the actual material to be used, and under conditions of actual practice. This point has been stressed by various workers on textiles and wood preservation.

Standardisation of Antiseptics.—As pointed out above, the biological standardisation of an antiseptic can only be very approximate. The " phenol coefficient " should only be regarded as a very rough guide, and may not give a true indication of the relative value of antiseptics under all conditions. Chemical standardisation is even more unreliable; the mercury content of an antiseptic conveys very little information as to its toxicity unless the exact constitution is known. It is often proposed that makers of proprietary antiseptics should label their products with an exact description of their chemical constitution. Apart from the difficulty of enforcing this, it would be of little benefit to the practical man. A much more essential reform is that makers of proprietary substances should be compelled to indicate any change of constitution by a corresponding modification in the name of the product. Otherwise recorded work becomes valueless.

REFERENCES

British Standards Institution (1934): Specification No. 541; (1938): Specification No. 808.

Burgess, R.: (1934): J. Soc. Dyers Col., **50**, 138.

Chapin, P. (1902): Flora, **91**, 348.

Fargher, R. G., Galloway, L. D., and Probert, M. E. (1930): J. Text. Inst., **21**, 245T.

Galloway, L. D. (1935): J. Text. Inst., **26**, 123T.

—— (1934): Manf. Chemist, **5**, 233.

Gibson, J. (1936): Chem. and Industry, **55**, 107.

International Critical Tables (1926-33). London.

Klotz, L. J. (1936): Hilgardia, **10**, 27.

Medical Research Council (1931): System of Bacteriology, Vol. 9. H.M. Stationery Office.

Morris, L. E. (1926): J. Text. Inst., **17**, 1T.

Patterson, T. C., and Frederick, R. C. (1931): Analyst, **56**, 93.

Rideal, S. (1921): Chemical Disinfection and Sterilisation. London.

Thaysen, A. C. (1938): J. Hyg., **38**, 558.

Tomkins, R. G. (1929): Proc. Roy. Soc., B**105**, 375.

—— (1937): Trans. Brit. Mycol. Soc., **21**, 126.

Wilson, R. E. (1921): J. Ind. Eng. Chem., **13**, 326.

PART TWO

CHAPTER IX

FOOD INDUSTRIES

FOOD SPOILAGE

ALMOST all foodstuffs are liable to attack by micro-organisms. According to circumstances the damage may merely result in slight wastage, may cause total loss, or may lead to serious illness if the food is consumed. Mould fungi are able to develop in the presence of comparatively little moisture; they do not, however, give rise to any danger of food poisoning. Bacteria require damper conditions for their development, and owing to their rapid growth they can cause serious loss in a very short time. Useful textbooks are those of Marshall (1921), Smyth and Obold (1930), and Tanner (1932).

Preservatives.—In former days the producer attempted to minimise such losses by the addition of antiseptics, or "preservatives," to the food product. Benzoic acid, boric acid, formaldehyde, and other chemical substances, all helped to check decomposition, especially by bacteria. The objections to the use of preservatives gradually became manifest, so that the label "free from preservatives" became regarded as a mark of quality. As a result of the Government Committee's Report of 1924, new food regulations were made which practically prohibit the use of antiseptics, with a few exceptions, such as small quantities of sulphurous acid in fruit juices or meat products.

The reasons for discouraging the use of preservatives are:

(1) That nearly all these substances are to some extent harmful to the human system.

(2) That fresh food kept fresh by cool storage, rapid transit, and cleanliness in handling, is richer in vitamins, and is in all ways preferable to stale or dirty food made to appear fresh by chemical means.

(3) That in most cases modern facilities for refrigeration and

rapid transport have made the use of preservatives unnecessary.

Useful Organisms.—Not all micro-organisms are harmful to the food industries. The utilisation of bacteria and fungi in the preparation of bread, vinegar, and fermented products will be dealt with in Chapter X. Yeast is in itself a valuable food product. Other instances of beneficial organisms will be given below, especially in connection with dairy products.

DAIRY PRODUCTS

Milk.

Milk, besides being an ideal food for mammals, is also an extremely favourable medium for the growth of bacteria. The fact that today cleaner milk is obtainable than ever before is strong evidence that the present regulations against preservatives can be applied even in the most difficult cases.

Milk contains very few bacteria as it leaves the cow, most of the infecting organisms entering later—from the cow, the milker, the utensils, and dust and air contaminations at all subsequent stages. Much may be done to minimise infection by scrupulous cleanliness, involving washing of the cow's body (tail, flanks, and udder), the milkers' hands and milking stools, and by the sterilisation of all utensils; by maintaining an atmosphere as free from dust as possible; and by cooling the milk as soon as it is drawn. Live steam, boiling water, or hypochlorite solutions are helpful in securing the requisite aseptic condition of utensils in dairy or factory; steam sterilisation is best.

The number of bacteria soon increases with great rapidity, and at ordinary temperatures (15°-30° C.) the lactic acid producer *Streptococcus lactis* usually soon predominates. "Sour" milk of this type may not be very palatable, but the souring organisms are harmless, and the acidity prevents the development of proteolytic bacteria that would give the milk a still more objectionable smell and taste. After thirty-six to forty-eight hours' growth, a firm, non-contracting, sharp-tasting curd is produced. At higher temperatures (30°-40° C.) rod-shaped lactic acid producers, such as *Lactobacillus casei*, develop, whilst bacteria of the *coli-aerogenes* group may grow from 15°-40° C. The latter ferment the lactose with production of gas and give rise to disagreeable taste and odour.

The curd which they produce extrudes much whey, and is contracted and gassy. At much higher temperatures still (above 45° C.), any microbial action that may occur is due chiefly to thermophilic bacteria—*e.g.*, *Lactobacillus thermophilus*.

The lactobacilli are able to grow after the acidity has checked the other bacteria, and in the case of some strains may increase the acidity to as much as 4 per cent. lactic acid.

Defects in Milk may be due to a number of causes. Among the defects due to bacteria are:

(1) Gassiness, due to lactose-fermenting organisms such as *Bact. coli* and *Bact. aerogenes* and less frequently by species of *Clostridium* and yeasts.

(2) Ropy milk, due to capsule-forming bacteria—*e.g.*, *Alcaligenes viscosus* (*Bact. lactis viscosus*), *Str. cremoris* (at low temperatures) and types of *Bact. aerogenes*.

(3) Defects involving the taste of the milk (soapy, turnipy, caramel and bitter tastes), its colour, or its appearance and nature, as is often the case with milk drawn from cows suffering from mastitis and sometimes udder tuberculosis.

Oospora (*Oidium*) *lactis* and yeasts invariably grow on the surface of milk which has been kept too long. The former attacks protein and utilises lactic acid as a source of energy and carbon; eventually its action makes conditions suitable for proteolytic bacteria.

Many pathogenic bacteria can thrive in milk, and milk may be the vehicle of infection for man, pigs, calves, and other animals fed upon it. The most discussed species is that causing bovine tuberculosis, and with regard to this it is claimed that milk from infected cows leads to human infection, particularly of children, the chief consumers of milk. Hence arises the demand for pasteurisation. *Mycobacterium* (*Bact.*) *tuberculosis* itself does not multiply in milk, but can survive in it. Of other pathogenic bacteria and viruses some—*e.g.*, those causing mastitis, contagious abortion, brucellosis and foot-and-mouth disease—are derived from a diseased condition of the cow, whilst others—*e.g.*, those associated with diphtheria, typhoid, scarlet fever, septic sore throat, and infantile paralysis—originate from infected humans or human "carriers." Numerous epidemics of the latter group of diseases have been traced to the infection of milk supplies (*cf.* Chapter XII.).

A large part of the milk supply is now handled in the dairy

factory. Here the milk, which is derived from many farms, is bulked, and hence in the interests of public health has to be pasteurised or otherwise treated to render harmless any pathogenic organisms present. In essence a typical modern method of processing is as follows: The milk is taken from the farms to the country depots, cooled to 40° F. (4·4° C.), and transported in insulated tanks to the town factory. Here it is bulked, preheated to about 98° F. (36·7° C.), and gross dirt removed by clarifying machines or filter cloths. The milk is then heated to 145° F. (62·8° C.) and " held " at that temperature for thirty minutes, this process constituting pasteurisation. It is then rapidly cooled to 40° F. (6·4° C.), filled into bacteriologically clean bottles, and held in cold storage until distribution to the customer. In some factories the " holding " is effected in the bottle, a process which is claimed to prevent contamination subsequent to pasteurisation. Thermophilic bacteria that give pin-point colonies on milk-count plates may develop at the temperature of pasteurisation, whilst thermoduric (chiefly spore-forming) and certain lactic and coliform strains may survive.

The arguments commonly employed against pasteurisation are mainly that (1) it affects the taste and food value—*e.g.*, vitamin content—of the milk; (2) it removes the stimulus to absolute cleanliness in milk production; and (3) exposure to tuberculosis infection of such a mild type may not be such a bad thing after all, and may stimulate in the consumer an immunity against a more severe attack.

Medical opinion in general is that although pasteurisation is not a substitute for clean production, yet it is at present the only method of safeguarding the milk supply in large towns (Milk Commission Report, 1933). Raw milk may be pasteurised only if it is of good quality, and pasteurisation to be effective must be carefully controlled. Re-pasteurisation is a wholly undesirable process. In the " Flash " method of pasteurisation the milk is heated rapidly to 170°-175° F. (76·7°-79·4° C.), kept there for some seconds to one minute, and then cooled. This method, although not officially recognised in Britain, is largely used in America and elsewhere. Possible disadvantages are the uncertainty that all harmful organisms are killed off, reduction in the cream line, doubtful keeping quality, and trouble in the machinery due to the drying of

milk against steam-heated surfaces. Milk is also sometimes " sterilised "; this process involves heating good quality milk in bottles in ovens at 100° C., keeping it at that temperature for thirty minutes. Even during such treatment spore formers may survive and develop subsequently.

Methods of Testing Milk.—At present methods of testing, cleanliness and grading are based largely on bacteriological tests. Until recently the technique commonly employed consisted of ascertaining the bacterial count as indicated by the number of colonies developing on agar, and the presence or absence of the *coli-aerogenes* group producing acid and gas in bile salt lactose medium in two days at 37° C. These media are described in Chapter VI. They are inoculated with 1 ml. of sterile tap water or Ringer dilutions corresponding to the equivalent of $\frac{1}{10}$, $\frac{1}{100}$, and $\frac{1}{1000}$ ml. of milk, and, in the case of the bile salt medium, 1 ml. of the original milk also (see Chapter V.). The milk is first well shaken to distribute the fat globules and micro-organisms. Counting of the colonies on the agar plates is facilitated by the use of the standard illuminated counting chamber described by Mattick and Hiscox (1933) and a tally counter.

Owing to the general unsuitability of the plate count method for milk, its replacement by a simpler test, the *Methylene Blue Reduction Test*, has been strongly advocated (Wilson *et al.*, 1935). This measures the rate of reduction of methylene blue added to milk. It is quicker, simpler and cheaper than the plate count, and is claimed to be more accurate and sensitive. It also affords a good index of the keeping quality of milk. From the beginning of 1937 the reduction test has become recognised as a standard test for milk examination.

One ml. of methylene blue solution (one tablet of approved methylene blue in 800 ml. of distilled water) is added to 10 ml. of milk in a sterile tube fitted with a sterile rubber cork. After mixing, the tube is incubated in darkness at 37° C., and inverted once every half-hour. The end point is indicated by the decolorisation of the dye, a 5 mm. surface rim of colour being ignored. The greater the bacterial content of the milk, the more rapid is the loss of blue colour. Milk, to be satisfactory, must not decolorise the methylene blue within 4½ hours in summer and 5½ hours in winter. Limitations of the reduction test are suggested by Malcolm and Leitch (1936).

The fact that *coli-aerogenes* organisms grow readily in milk is one of the several reasons why the so-called presumptive coliform test is of limited use. It may, however, serve to indicate uncleanly methods of milk production, and can be advantageously applied where industrial aspects of dairying are concerned. It is retained along with the reduction test as the standard routine method for graded unpasteurised milk, while the plate count method is used for testing pasteurised milk (see Min. of Health Memo. 139/Foods, 1937).

An alternative enumerative method to the plate count is the direct microscopic count or Breed method. Here 0·01 ml. of milk is spread evenly over an area of 1 sq. cm. etched on a clean sterile glass slide. The film is air-dried, fixed for one minute in methyl alcohol, and stained fifteen to thirty seconds with Newman's stain (*cf.* Chapter VI.). The preparation is then washed, dried, and examined under the oil immersion lens. The average number of organisms or clumps per ml. is obtained by the examination of an appropriate number of fields, and hence by calculation the number of organisms per ml. of milk. Advantages of this method over the plate method are rapidity, cheapness, ability to enumerate the majority of organisms present, and independence of culture media upon which certain bacteria may fail to grow. Disadvantages of the method are that it does not distinguish clearly between dead and living bacteria (hence it cannot be used for pasteurised milk), that not all the organisms take the stain, and that it can only be used for grading milk with a high bacterial count.

For the detection of heated milk, or the presence of raw milk in heated milk, tests have been devised depending on the destruction by heat of certain enzymes. A useful one is the phosphatase test, that distinguishes between raw and pasteurised milk, and serves as a check on the efficiency of pasteurisation (Kay and Graham, 1935; Kay *et al.*, 1939).

Grading of Milk.—The Draft Milk (Special Designations) Order, 1936, lays down four grades as follows:

A. *Raw Milk.*—(1) "Tuberculin Tested." Animals must not react to tuberculin tests carried out within specified intervals.

(2) "Accredited." Animals must be certified every three months as showing no evidence of disease likely to injure the milk.

Both grades must satisfy the methylene blue reduction test

as outlined above, and must not contain coliform bacteria in $\frac{1}{100}$ ml.

B. *Pasteurised Milk.*—Milk that has been held at 145° F. (62·8° C.) for thirty minutes.

(3) "Tuberculin Tested (Pasteurised)." Prepared from "tuberculin tested" milk.

(4) "Pasteurised." Prepared from good quality milk of no special designation.

Grade 3 must not contain more than 30,000 and grade 4 100,000 bacteria per ml.

The conditions under which the milk should be sampled and kept before testing are given in Min. of Health Memo. 139/Foods, 1937.

For additional details regarding the microbiology of milk, reference may be made to Orla-Jensen (1931) and Chalmers (1935).

A standard method for the bacteriological control of the efficiency of milk-bottle washing is put forward by Mattick and Hoy (1937), who also discuss the bactericidal efficiency of hot alkaline detergents.

Cheese.

Both fungi and bacteria play an important part in producing the characteristics of the many varieties of cheese.

The formation of curd is usually secured by the use of rennet, but in certain types it is brought about by natural souring of the milk by lactic bacteria. In either case, lactic and other bacteria or mould fungi take part in the subsequent ripening.

Rennet curd cheese may be either (1) hard, *e.g.* Cheddar, in which the ripening is uniform and conditions anaerobic, or (2) soft, *e.g.* Camembert, in which the chief ripening agents grow on the surface, their enzymic action extending inwards. Certain types of hard cheese (Roquefort, Gorgonzola, Stilton) show mottling or blue veining due to the growth of *Penicillium roqueforti*, which develops in the partially aerobic environment following shrinkage of the curd, and, attacking especially the fats, produces caproic, caprylic and other acids, and imparts a characteristic flavour. Its activity is sometimes increased by pricking.

In the production of hard cheese a "starter" of *Streptococcus* spp. is added to the milk. This leads to a rapid development of acidity which favours the coagulating action of the rennet

and also overpowers the growth of undesirable organisms. The milk may previously be given flash pasteurisation at 150°-165° F. (65·6°-73·9° C.) to ensure uniformity. During the course of ripening the streptococci are superseded by lactic rod bacteria, especially *Lactobacillus casei*. These bacteria thrive under the anaerobic conditions of the cheese and resist the salt.

Soft rennet cheese have a higher moisture content than hard cheese. They are at first very acid, but the later proteolytic action of surface fungi such as *Penicillium camemberti* and *Oospora lactis* reduces the acidity and modifies the flavour. Careful control of the organisms is necessary to produce satisfactory results, and the cheese has poor keeping quality, since there is not sufficient acidity to check putrefaction. One well-known soft cheese employs a starter of *Lactobacillus bulgaricus* and *L. acidophilus*, which gives a very high lactic content. Yeasts are of common occurrence in old soft cheese.

Defects in Cheese.—The successful ripening of cheese involves careful control of temperature, moisture content, and pH. The use of a good starter is essential. Among the defects due to micro-organisms the following may be mentioned:

(1) Gassiness, holes being due to organisms of the *Bact. aerogenes* type, which in turn result from unclean milk production. The trouble may be checked by pasteurisation of the milk.

(2) Undesirable flavours caused by the development of putrefactive bacteria during the later, less acid, stages of ripening.

(3) Discoloration—*e.g.*, "rusty" discoloration in Cheddar due to *Lactobacillus rudensis*.

(4) Slow acid development, the growth of the lactic bacteria being retarded by some inherent condition of the milk; a feature common where the cows are suffering from mastitis. For an account of mastitis in relation to cheese-making see Davis and McCemont (1939).

(5) Mould development of the wrong type. *Scopulariopsis brevicaulis* frequently overgrows Camembert, and produces a strong ammoniacal flavour. Stored cheese sometimes suffers from surface moulds, and methods of treatment such as dipping in paraffin wax, coating with a thin film of tasteless oil, butter and grease proof paper, etc., depend on the exclusion of air.

Examination of Cheese.—For microscopic examination a thin smear of the cheese may be de-fatted and stained; in hard cheese the bacteria will be seen in colonies. Or the

cheese may be ground with water and examined as for milk, but with the substitution of whey peptone agar (whey 1,000 ml., peptone 10 grms., sodium chloride 5 grms., powdered agar 15 grms., pH 7·0) for standard milk agar.

For further information on cheese see Orla-Jensen (1931) and Davis (1935).

Butter.

During the separation of the cream large numbers of bacteria are carried over by the fat globules, and hence the bacterial concentration in the cream is higher than in the milk from which it came. These organisms, during the ripening process, contribute largely to the characteristic butter flavour, and during churning most of them are thrown out with the buttermilk, the flavour being absorbed by the butter fat.

Butter may be made from sweet cream or from sour cream. The latter method is more common in northern Europe, and produces butter with a better keeping quality. The cream is pasteurised at 75°-85°, and inoculated with a starter (usually containing *Str. cremoris* and *Leuconostoc dextranicum* [*Str. paracitrovorus*], which are aroma-producing lactic types).

The flavours of butter resulting from microbiological action are due to volatile acids and other substances—*e.g.*, diacetyl—produced from citric acid originally in the cream, and from lactates.

Defects.—Bacterial defects during the manufacture of butter include:

(1) Slime production, often caused by certain types of lactic bacteria.

(2) Small gas holes, caused by the growth of anaerobic butyric bacteria.

(3) Cheesy taste, due to certain rod-shaped lactic organisms.

(4) "Yeasty" flavour, caused by yeast growth.

In storage, butter may suffer deterioration or discoloration due to *Micrococcus* spp., *Pseudomonas* (*Bact.*) *fluorescens*, *Serratia marcescens* (*Bact. prodigiosum*), and to mould fungi, including *Torula* spp. The moulds have been studied by various workers; Vernon (1935) reports blackish spots due to *Cladosporium* and *Stemphylium*, spreading brown stains due to *Phoma* and *Alternaria*, green surface growths of *Penicillium* and *Aspergillus*, and bright red stains caused by *Fusarium*. Nutrient matter may sometimes come from the wood of the

packing boxes, and the softening treatments (based on glycerol) given to parchment covers may enhance mould growth.

Micro-organisms grow in the larger water droplets. They attack both fats and casein. Fatty acids and esters are formed, resulting in "rancidity." Control of moulding and rancidity due to micro-organisms is obtained by pasteurisation, followed by the use of a reliable starter, by sterilisation of wooden and other utensils, by careful attention to the purity of the water and salt, by thorough washing of the butter, and by the exclusion of air (storage in bulk, or use of clean sterile wrappers).

Bacteriological examination is carried out by making a suspension of the butter in warm diluent and plating out as for milk, using warm apparatus.

Other Dairy Products.

Condensed Milk.—Sweetened condensed milk is protected from attack by its sugar content of 30 to 40 per cent., and is therefore not sterilised. Occasionally some yeast development occurs, leading to swelling of the cans, or moulds such as *Aspergillus repens* lead to the formation of "buttons."

Unsweetened evaporated milk, on the other hand, is steam sterilised at the time of canning. Deterioration by micro-organisms—usually spore formers—occasionally occurs, and leads to coagulation or peptonisation of the proteins, or to gas formation by *Clostridium* spp.

Dried Milk is immune from attack unless stored in a moist atmosphere, when it may develop mould growth.

The bacteriology of condensed and dried milk is discussed by Allen (1932) and Nichols (1936); for spray-dried milk see Crossley (1938).

Ice Cream.—Bacterial growth in ice cream is not uncommon, and has frequently led to food poisoning and disease. Pasteurisation of the cream, the use of pure ingredients, and clean conditions of production are essential.

Fermented Milk Drinks will be briefly discussed in the next chapter.

Margarine is a blend of vegetable and/or animal fats, to which a small quantity of separated milk is added. The milk is often inoculated with a starter to develop a certain degree of acidity and flavour.

MEAT, MEAT PRODUCTS, AND FISH

The muscle tissue of healthy, fresh-killed animals and fish is sterile, but bacteria develop rapidly on the surface and if conditions are favourable penetrate into the tissues. Species of *Clostridium*, etc., penetrate the bowel wall a short time after the death of the animal, and, unless bacterial action is arrested by some means, putrefaction follows. Bacterial numbers increase rapidly on the cut surface of meat, often rising to millions per gramme, but penetration between the muscle fibres is generally not rapid. Putrefaction occurs more rapidly in the case of fish. Game decomposes less rapidly than ordinary meat, and can safely be hung for long periods.

True putrefactive spoilage is due chiefly to the action of anaerobic bacteria—*e.g.*, *Cl. sporogenes* and *Cl. putrificus*, leading to malodorous by-products of the indole type, and poisonous substances derived from amino-acids. On the other hand, aerobic bacteria of the *Bac. subtilis* group, and facultative anaerobes—*e.g.*, *Bact. coli* and *Proteus* groups—all set up initial changes which may be considered as digestive and preparatory to true putrefaction. Attempts to correlate the number of bacteria or of specific types with the condition of the meat have so far failed to give convincing results.

Meat spoilage may also be caused by the formation of bacterial slime on the surface, this being enhanced by the extent of initial infection and the temperature of storage. The minimum temperature of growth of the causal organisms, which are species of *Achromobacter*, is – 3° C. To prevent bacterial growth storage below this temperature is necessary, and hence the meat is frozen. For a detailed account of the bacteriology of meat see Haines (1937).

Meat in cold storage is subject to mould spoilage (Brooks and Hansford, 1923). Frozen meat and poultry are liable to develop "Black Spot" due to *Cladosporium herbarum* when kept at – 2° to – 5° C. "White Spot" is caused by *Sporotrichum carnis*, and green patches by species of *Penicillium*. The formation of greyish "whiskers" is due to species of *Mucor* and *Thamnidium*. The minimum temperature for growth of these fungi is from – 5° to – 7° C. Prevention of all mould growth is obtained by storing in a frozen state at – 10° C. While mutton may be successfully transported from

overseas in a frozen condition, beef is preferably " chilled " at −1° C., when its " life " is limited to about one month. Moulds and bacteria are checked and the life of the beef thus doubled by adding 10 to 20 per cent. of carbon dioxide to the storage atmosphere: higher concentrations cause discoloration (Moran *et al.*, 1932).

Older methods for preserving meat are drying, salting and smoking: the last method is probably a combined effect of desiccation and the phenolic substances supplied by the wood smoke. The *curing* of meat is usually carried out by immersion in strong brine solution containing about 1 per cent. saltpetre or potassium nitrate. The osmotic tension of the cells is increased by the brine, giving a preservative effect; at the same time salt-tolerant denitrifying bacteria develop and reduce the nitrate to potassium nitrite. This substance has the effect of giving the meat a red colour. The process is somewhat difficult to control, and now that the mechanism of the action is known it is common in some countries to add nitrite direct to the brine, instead of relying on the bacterial reduction of nitrate.

The bacterial content of prepared meat products—*e.g.*, brawn, potted meats, and sausages—varies very considerably. Cooking immediately after preparation—if properly carried out—destroys most of the micro-organisms, but bacteria which survive cooking, or bacteria and moulds which subsequently gain access, will develop if the temperature allows. Inefficient sterilisation of canned meats may also lead to trouble, the sporing anaerobes and also *Bact. coli* and *Bact. aerogenes* all being capable of giving rise to " blown " cans. Sporing aerobes will not develop unless air is allowed to enter.

White fish is often gutted and stored with salt in piles before smoking. During this time it may develop red discolorations (" Pink Eye "); fish may also become discoloured in cold storage. The cause of the reddening is bacterial, chromogenic cocci and rods and *Pseudomonas fluorescens* being among the types that have been identified. Some of these organisms require a high salt content (16 per cent.) for their development, while all are salt tolerant (see p. 68). Non-chromogenic bacteria, though less obvious, are also present, and give rise to discoloration, softening, and a foul odour (Gibbons, 1933). Thaysen and Pentelow (1936) have shown

that the earthy flavour sometimes noticeable in fresh-water fish may be due to contamination of the streams with odour-producing actinomycetes.

Eggs.

Not more than 10 per cent. of fresh eggs contain bacteria, the organisms being found chiefly in the yolk. Bacterial decomposition, when it occurs, is not due to these organisms, which do not belong to proteolytic or fermentative groups. The egg shells, however, are usually contaminated, and if the protective gelatinous coating is soiled or removed by rough handling bacterial penetration may occur, especially in damp storage. The eggs of hens infected by pullorum disease (*Salmonella pullorum*) may be infected in the ovary, and the resulting chicks may die in the shell, or show the characteristic symptoms of bacillary white diarrhœa soon after hatching.

Coloured rots due to *Pseudomonas* spp. are described by Miles (1937).

A further type of egg deterioration, often very troublesome, is caused by moulds. These readily develop in a humid atmosphere. During storage of eggs the relative humidity of the atmosphere should therefore be kept below the minimum for mould growth (about 85 per cent. R.H.), but high enough to prevent undue evaporation of moisture from the eggs (about 70 per cent. R.H.). Soiled eggs may develop moulds at a relative humidity lower than 90 per cent. Storage of eggs in an atmosphere containing 90 per cent. or more of carbon dioxide prevents mould growth.

Fruit and Vegetable Products.

The relations of micro-organisms to the living plant are discussed in Chapter XIII. After harvesting, fruits and vegetables are subject to deterioration by fungi and bacteria whilst in storage. Control by cold storage presents certain difficulties, since too low temperatures may affect the tissues adversely.

For the prevention of fruit decay, the most important factor is the avoidance of bruises or wounds through which the infecting organisms may enter. Wrapping in paper gives valuable protection from damage and helps to minimise infection. Chemically treated papers—*e.g.*, with iodine (Tom-

kins, 1934), diphenyl, etc.—may give additional protection in some cases. Of the many chemical treatments tried directly on the fruit, borax has given the most uniformly successful results. Nattrass (1935), however, reports good results with immersion of citrus fruits in 1 per cent. salicylanilide.

The so-called "gas storage" of fruit in atmospheres containing small proportions of carbon dioxide aims at the control of the respiratory activities of the fruit rather than any direct effect on the rot-producing organisms. The addition to the atmosphere of minute amounts of certain volatile substances —notably acetaldehyde and ammonia—has been suggested as a method of control (Tomkins, 1932; Tomkins and Trout, 1931). So far the results are only partially successful, but the method offers a new line of attack on the problem.

The fungi causing rotting of fruits include many of the common moulds. *Penicillium* spp. are particularly common; *P. expansum* is a frequent species on apples, and *P. italicum* and *P. digitatum* on citrus fruits. *Mucor* and *Rhizopus* are common on soft fruits such as strawberries.

Fruit juices readily develop moulds, and a surface in contact with air should be avoided. Addition of the specified quantities of sulphurous acid gives some protection.

A method of preservation applicable to some fruits and vegetables is desiccation, followed by storage under dry atmospheric conditions. A modern development of cold storage is the "frozen pack."

Vegetables are even more susceptible than fruits to deterioration, conditions for bacterial growth being more favourable. The principle of preservation by pickling is the encouragement of a lactic fermentation that checks the growth of putrefying bacteria. Such materials are not immune from moulds—*e.g.*, *Oospora lactis*—and should be protected from mould spoilage by the exclusion of air. The question of pathogenic bacteria conveyed by vegetables is discussed in Chapter XII.

Tomato ketchup is particularly liable to spoilage by yeasts, moulds, and bacteria, and careful microbiological control is necessary during manufacture.

Canning.

The preservation of fruit, vegetables, and meat products by canning has now reached a high stage of efficiency; improvements have been introduced in methods of sterilisation, sealing, and lacquering, and the various factors to be considered for successful canning are now better understood. Fruit particularly lends itself to canning, the acidity of the fruit juices combined with the heat applied making sterilisation a simpler matter than in the case of meat and vegetable products. An interesting mould fungus recently discovered to be unexpectedly resistant to heat is *Byssochlamys fulva* (Olliver and Rendle, 1934), which has been a source of trouble in the canning of strawberries.

Imperfect sterilisation of meat and vegetable products may lead to the development of pathogenic organisms, the most deadly of these being *Clostridium botulinum*. Food poisoning will be more fully considered in Chapter XII.

Savage *et al.* (1922) demonstrated that absolute sterility is not necessary. A large proportion of sound cans contain living organisms, of types that do not develop under the conditions within the can. On the other hand, fermenting organisms in fruit, and gas-forming or proteolytic bacteria in meat, lead to spoilage of the product.

From the microbiological point of view, essential features for successful canning are soundness and freshness of the materials, the absence of leaks in containers, adequate sterilisation which will of course vary with the product, and bacteriological control at all stages of manufacture (Jones, 1937).

Cereals, Sugar, and Miscellaneous Products.

Grain.—Stored grain must be kept down to a certain limit of moisture content varying from 12 to 16 per cent. for various cereals, or mould fungi will develop and lead to mustiness and acidity. An additional safeguard is the exclusion of air as far as is practicable. Horses and pigs are stated to be more susceptible than ruminants to ill effects after feeding on mouldy grain. Poultry may contract aspergillosis. At higher moisture contents—say, 30 per cent.—bacterial growth is rapid. Milled wheat flour usually has a bacterial content of

10,000 to 500,000 per grm., the number diminishing on storage. Excessive infection of flour used for baking by organisms of the *Bac. mesentericus* type may lead to " ropy " bread.

Sugar.—*Aspergillus*, *Penicillium*, and other moulds, as well as yeasts and certain bacteria, may develop in the crude juices during the preparation of cane or beet sugar, causing loss by the inversion or total destruction of the saccharose. Among the bacterial species commonly present is *Leuconostoc mesenterioides*, which under neutral or alkaline conditions produces considerable quantities of mucus (see Chapter VII.). Sugar kept in damp storage is also subject to inversion due to mould growth on the bags. Slow growth of certain yeasts, and moulds of the *Asp. glaucus* group, can occur in syrups even up to 65 per cent. sugar content. Jams and lemon curd will mildew at the surface unless protected by waxed discs, or metal tops may be used and the surface layers sterilised by heat. A summary of the literature on the micro-organisms of cereals and sugar is given by Thaysen and Galloway (1930).

The " bursting " of chocolate creams is sometimes brought about by yeasts or bacteria (*Clostridium* sp.) fermenting the sugar, and may be prevented, according to Paine, Birckner, and Hamilton (1927), by partial inversion of the sugar, secured by the addition of saccharase (invertase). This enables a paste of higher density to be employed, and thus checks growth of organisms. Commercial preparations of saccharase, obtained from yeast, are also used in the confectionery industry to prevent the crystallisation of sugar. " Bloom " on chocolates, often mistaken for mildew, is caused by separation of the fats at the surface, and may be to some extent prevented by the addition of lecithin to the cocoa butter used.

Nuts frequently develop mould spoilage. The infection, usually *Aspergillus* and *Penicillium*, enters through cracks in the shells, and careful handling is therefore desirable.

Copra is also liable to develop moulds if stored damp. The rancidity of coconut oil is, in some cases, due to *Penicillium* spp.

Cocoa Beans are given a natural fermentation, mainly to facilitate removal of the pulp (Knapp, 1937). If allowed to retain over 9 per cent. moisture, moulds will develop. Cocoa made from affected beans will have a musty flavour.

Fungi as Food.—The utilisation of mushrooms and other

" higher " fungi for human consumption requires no comment. A more recent development is the use made of yeast in food preparations; a purely vegetable extract which is almost indistinguishable from meat extract may be prepared from yeast. Yeast for food has been produced by fermentation of sugar-containing solutions with added ammonium salts obtained from atmospheric nitrogen, as a source of nitrogen. Thus atmospheric nitrogen can be synthesised into an organic nitrogenous foodstuff within two or three days—a process that makes the ordinary methods of agriculture appear slow by comparison.

The possibility of using mould fungi as animal foodstuffs is also being considered by research workers in America and Japan.

REFERENCES

ALLEN, L. A. (1932): Hannah Dairy Research Inst. Bull., No. 3. Glasgow.

BROOKS, F. T., AND HANSFORD, C. G. (1923): D.S.I.R. Food Invest. Board Spec. Report No. 17. H.M. Stationery Office.

CHALMERS, C. H. (1935): Bacteria in Relation to the Milk Supply. London.

CROSSLEY, E. L. (1938): Soc. Agric. Bacteriologists. Abst. of Proceedings.

DAVIS, J. G. (1935): Chem. and Industry, **54**, 631.

—— AND MCCEMONT, J. (1939): J. Dairy Res., **10**, 94.

GIBBONS, N. E. (1933): Prog. Rep. Atlantic Biol. Sta., Fish Exp. Sta., No. 9, 5.

HAINES, R. B. (1937): D.S.I.R. Food Invest. Board Spec. Rep. No. 45. H.M. Stationery Office.

JONES, O., AND JONES, T. W. (1937): Canning Practice and Control. London.

KAY, H. D., ASCHAFFENBURG, R., AND NEAVE, F. K. (1939): Tech. Communication No. 1, Imperial Bureau of Dairy Science, Shinfield.

—— AND GRAHAM, W. R. (1935): J. Dairy Res., **6**, 191.

KNAPP, A. W. (1937): Cacao Fermentation. London.

MALCOLM, J. F., AND LEITCH, R. H. (1936): Scottish J. Agric., **19**, 321.

MARSHALL, C. E. (1921): Microbiology. London.

MATTICK, A. T. R., AND HISCOX, E. R. (1933): J. Sci. Instruments, **10**, 373.

—— AND HOY, W. A. (1937): Bottle Washing and Bottle-Washing Machines. N.I.R.D., Reading.

MILES, A. A., AND HOLMAN, E. T. (1937): J. Hyg., **37**, 79.

MILK COMMISSION REPORT (1933): Investigation on Complaints . . . as to the Operation of the Milk Marketing Scheme. H.M. Stationery Office.

MINISTRY OF HEALTH (1937): Memo. 139/Foods. Bacteriological Tests for Graded Milk. H.M. Stationery Office. London.

MORAN, T., SMITH, E. C., AND TOMKINS, R. G. (1932): J. Soc. Chem. Ind., **51**, 114T.

NATTRASS, R. M. (1935): Cyprus Agric. J., **30**, 84.
NICHOLS, A. A. (1936): J. Soc. Chem. Ind., **55**, 78T.
OLLIVER, M., AND RENDLE, T. (1934): J. Soc. Chem. Ind., **53**, 166T.
ORLA-JENSEN, A. D. (1931): Dairy Bacteriology. London.
PAINE, H. S., BIRCKNER, V., AND HAMILTON, J. (1927): Ind. Eng. Chem. **19**, 353.
SAVAGE, W. G., HUNWICKE, R. F., AND CALDER (1922): D.S.I.R. Food Invest. Board Spec. Report No. 11.
SMYTH, H. F., AND OBOLD, W. F. (1930): Industrial Microbiology. Baltimore.
TANNER, F. W. (1932): Microbiology of Foods. Illinois.
THAYSEN, A. C., AND GALLOWAY, L. D. (1930): Microbiology of Starch and Sugars. London.
—— AND PENTELOW, F. K. T. (1936): Ann. Appl. Biol., **23**, 99, 105.
TOMKINS, R. G. (1932): Proc. Roy. Soc., B**111**, 210.
—— (1934): J. Pomology, **12**, 311.
—— AND TROUT, S. A. (1931): *Ibid.*, **9**, 257.
VERNON, T. R. (1935): J. Dairy Res., **6**, 154.
WILSON, G. S., *et al.* (1935): Medical Research Council. Special Report Series—No. 206, The Bacteriological Grading of Milk. H.M. Stationery Office.

CHAPTER X

THE FERMENTATION INDUSTRIES

THE word fermentation in its strict sense implies a decomposition process due to micro-organisms, which is accompanied by frothing due to the evolution of gas. As commonly used, however, the term is also applied to processes in which no gas is evolved; this is a convenient practice to adopt, since there is no sharp dividing line between slow gas production and no gas production at all.

Alcoholic Fermentation.

The fermentation reaction of most importance is that leading to the production of alcohol from sugars through the agency of yeasts, and it is on this that the attention of practical men and chemical investigators has been principally focussed. Pasteur showed that it was essentially a biological process, and concluded that fermentation was a kind of anaerobic respiration, "the consequence of life without air." Later workers questioned this conclusion, and Buchner showed that an enzyme extract obtained from yeast cells could carry out the conversion of sugar to alcohol in the absence of living matter. Fermentation of the latter type, however, is still in the nature of a chemical curiosity, and is not a practical process. Pasteur's dictum still stands as a general truth, for free aeration of a yeast fermentation results in a diminished rate of alcohol production. Aeration increases growth (a fact of which use is made when yeast and not alcohol is the main product desired), but a higher proportion of the sugar undergoes complete oxidation. Conversely, if certain of the mould fungi or "wild" yeasts that normally grow aerobically and completely oxidise sugars are made to grow submerged in sugar solution, they will produce appreciable quantities of alcohol.

The nature of the chemical processes occurring during alcoholic fermentation has been briefly discussed in Chapter VII.,

where it is pointed out that a series of overlapping reactions and by-products are involved. The equation $C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$ serves to indicate the general course of a yeast fermentation, but by modification of the conditions other products such as glycerol may be made to predominate. Moreover, a small proportion of the sugar is always used up in oxidation to secure energy and in building up the rapidly multiplying yeast cells.

Particular fermentations are often specified in a very loose manner. Thus in a " yeast fermentation " yeast is the agent; in a " cellulose fermentation " cellulose is the material decomposed; and in a " vinegar fermentation " vinegar is the substance produced.

Industrial fermentations are most conveniently carried out in a large vat or special fermentation vessel, and this implies the use of an organism that can act under anaerobic conditions. Micro-organisms that require an ample air supply, such as acetic bacteria and the mould fungi, must have special arrangements to secure this. In the case of mould fermentations, shallow trays are often used to contain the fermentable liquor, introducing fresh complications as regards the exclusion of infection.

Successful fermentation depends on a number of factors. The mash must be suitable in concentration, reaction, nutrient value, and temperature. Failure may be due to the unsuitability of one or more of these conditions, to some inhibiting substance, or to infection by undesirable organisms; to detect the source of the last it is essential to take samples periodically at all stages of the process, for microscopic examination and plating. The extent to which slight infection affects the fermentation will, of course, depend on the properties of both the fermenting organism and the infection.

Raw Materials for Alcoholic Fermentation.—Dextrose and levulose are readily fermented by yeasts, as are in most cases the disaccharides saccharose and maltose. The polysaccharides starch and cellulose must first be broken down to hexoses by enzymic or acid hydrolysis. Pentoses are not fermentable by yeasts, although some years ago a commercial enterprise was founded on the assumption that they could be so fermented.

In technical practice the chief sources of fermentable

materials for alcohol production are molasses, starch in the form of grain or potatoes, and to a lesser extent sugar beet and various fruit juices. When starchy materials are employed the usual source of saccharifying enzyme is barley malt; this is partly germinated barley seed which has been heated to a temperature sufficient to check further growth, but not sufficient to destroy the enzyme that has developed during germination.

An alternative source of amylase is certain mould fungi, notably species of *Aspergillus* and *Mucor*. *Asp. oryzæ* and related organisms are commonly used in the Far East for the preliminary hydrolysis of rice—*e.g.*, for the preparation of the Japanese drink *saké*. Certain mucors are also employed in the production of industrial alcohol from starchy materials, one of the best known of such processes being the Boulard process, for which various patents have been taken out. Briefly, the procedure consists in (i.) a preliminary soaking of the grain in dilute acid, say 0·8 per cent. H_2SO_4 at 70° C.; (ii.) sterilisation by heat, cooling, and inoculation with a pure culture of a suitable Mucor species; (iii.) incubation for about twenty-four hours, during which time air is blown through, and the developing mould hyphæ permeate the mash; a yeast inoculant is then added to ferment the sugar which is being formed, and incubation is continued until fermentation is complete. Success depends on strict control of temperature, pH, and the purity of the cultures used.

This type of combined saccharification-fermentation process has certain advantages, but its development in this country has been checked by the nature of the Excise regulations, which require a record of the specific gravity of the saccharified mash before fermentation.

Brewing.

Many different types of beer have been developed, and the processes of their manufacture naturally vary in detail. In general, the principal stages are as follows:

(i.) **Malting.**—Barley is steeped in water, and the damp grain then allowed to germinate either on the malting floor or in pneumatic drums. During germination the starch saccharifying enzyme diastase develops. Growth is checked at

a suitable stage by *kilning*; the drying must be done within suitable temperature limits in order not to destroy the enzyme, and the lower the temperature used, the lighter in colour is the malt. In malts cured at high temperatures (88°-105° C.) much of the amylase is destroyed, but such malts are added in the manufacture of stouts and dark beers to give colour and flavour.

(ii.) **Preparation of Wort.**—The malt, after storage in air-tight bins, is ground and steeped in water at 60°-65° C. At this point unmalted grain (not necessarily barley) and other carbohydrate additions may be made. The mashing process is continued for about two hours until all the starch is transformed into maltose and dextrins, the liquid is run off, and the mash is further extracted ("sparged") with hot water until the extract reaches the required specific gravity. The resulting *wort* is boiled with hops (which provide a bitter flavour, and also help to check the growth of infecting bacteria), and is then strained, cooled and run into fermentation vessels. In certain breweries, particularly in Belgium, it is customary to acidify the wort before boiling, by allowing the development of *Lactobacillus delbrückii*.

(iii.) **Fermentation.**—Various yeasts are used for the brewing of different beers, but usually a top or bottom strain (or mixture of strains) of *Saccharomyces cerevisiæ* is employed. The inoculant is either obtained from a previous successful fermentation, or worked up from pure pressed yeast by successive additions of sterile wort. The initial pH of the wort should be about 5·0. Fermentation in the vats is carried on for about five days at 15·6°-18·3° C., and the fermented liquor is then run off ("racked") into casks.

(iv.) **After-treatment.**—The liquor is kept in casks for an appropriate time for the slow secondary fermentation to continue. It is later filtered or clarified by the addition of isinglass, this sometimes being first dissolved in sulphurous acid. When beer is bottled it is charged with carbon dioxide, which may be collected from the fermentation process. In casked beer the carbon dioxide is retained by bunging the cask before secondary fermentation, which is sometimes enhanced by the addition of a little glucose, is complete. An average ale contains 6 to 8 per cent. of alcohol by volume.

Defects due to Micro-organisms.—Brewing yeasts always con-

tain a certain percentage of bacteria, usually non-motile rods. Some of these do no harm, but certain types—notably *Lactobacillus* (*Saccharobacillus*) *pastorianus*—give a bitter flavour to the beer (Shimwell, 1935-36). Lactic acid-forming cocci are also a cause of cloudiness and loss of flavour (Shimwell and Kirkpatrick, 1939).

With modern methods of yeast control, bacterial troubles are less common than formerly, and defects when due to organisms and not purely chemical are usually attributable to " wild yeasts," various species of which may cause a bitter flavour or persistent turbidity.

Examination of Beer. — Careful microscopic control is advisable at all stages. Incubation of samples of beer at a higher temperature often hastens the development of infecting organisms, which can then be examined more conveniently.

Hops.—The antiseptic action of hops has already been referred to. For the evaluation of the antiseptic value of hop samples, the log-phase method devised by Walker (1931) may be used. The antiseptic potency is measured by its inhibiting effect on acid production by *Lactobacillus bulgaricus* grown in wort under strictly defined conditions.

Hops are subject to mildew and other fungal diseases in the field, and are also liable to mouldiness in damp storage.

For further details regarding brewing technique, see Luers (1929) and Hopkins and Krause (1937), and Laufer and Schwarz (1936).

Wine-making.

The term wine should only be applied to the fermented juice of the grape, although alcoholic drinks prepared from other fruit juices are often loosely called " wines."

In the making of wine the grapes are pressed, and the juice or " must " is allowed to settle, decanted, and then fermented. The types of yeast employed are strains of *Saccharomyces ellipsoideus*, and not *S. cerevisiæ* as used for brewing. The natural infection of the grapes may be relied on for the yeast inoculation, or more usually a pure culture or " starter " is added. It is usual to treat the crushed grapes with 75 to 100 parts per million of sulphur dioxide; this has little effect

on the growth of *S. ellipsoideus*, but checks the development of moulds, bacteria and other yeasts.

For different wines and in different countries many modifications exist. Red wines owe their colour to the skins of the crushed grapes. In "dry" wines fermentation of the sugars is practically complete, as opposed to "sweet" wines, in which the alcoholic fermentation is stopped at an earlier stage. Wine may be fortified by the addition of wine spirits. The alcohol content of wine varies from 7 to 16 per cent. by volume. Time of fermentation varies from three to fourteen days, and careful temperature control is essential. When fermentation is complete the wine is drawn off from the sediment, and cleared by some means appropriate to the particular kind of wine.

Defects due to Micro-organisms.—One of the chief essentials for success in wine making is the suppression of contamination, notably *S. apiculatus* (*Pseudosaccharomyces apiculatus*). The cells of this yeast are often lemon-shaped, and are only half as large as those of *S. ellipsoideus*. Other wild yeasts are *S. pastorianus* and *S. anomalus* (*Willia anomala*), the latter producing characteristic "bowler hat" endospores. *Mycoderma vini* forms a surface film that destroys flavour and reduces the alcohol content; it occurs in partly filled or poorly sealed containers. Other *Torula* yeasts (without endospores) also commonly occur.

Mould fungi, especially species of *Mucor* and *Penicillium*, are a frequent source of trouble on damaged grapes, on empty barrels and vats, and on corks, giving rise to a musty flavour. They may be controlled by the application of sulphurous acid. *Botrytis cinerea* is in some districts of France encouraged to grow on the grapes, since it leads to concentration of sugar in the juice; but it is on occasion capable of causing trouble.

Bacteria leading to defects in wine include a variety of types. The aerobic acetic acid bacteria cause souring of wines exposed to the air. Lactic and propionic bacteria, the long rods of the anaerobic "tourne" organism, mannitol-producing bacteria, micrococci, and various slime-producing organisms, all lead to loss of flavour. The remedy in all cases is adequate application of sulphur dioxide.

For the microscopic examination of spoiled wines it may be necessary to hand centrifuge the sample and prepare a slide

from the sediment. For an account of the technique of wine making see Cruess (1934).

Fermented Milk Beverages.

Many types of sour milk beverages are popular in parts of Central Europe and elsewhere. The drinking of sour milk as a means of prolonging life was advocated years ago by Metchnikoff, and it is still recognised that harmful putrefactive organisms in the intestine are suppressed by the presence of lactic acid organisms. It is sometimes claimed, however, that this result may be more simply obtained by the adoption of a milk diet without preliminary souring of the milk, bowel-resistant species of *Lactobacillus* developing naturally under these conditions.

Of the sour milks that contain at most only a little alcohol, the following may be mentioned. **Yoghurt,** a Bulgarian product, is soured by the bacterium *Lactobacillus bulgaricus,* which is active at 40° C. **Mazun,** an Armenian drink, is somewhat similar, and so is " **acidophilous** milk," produced commercially in England by inoculation of milk with *Lactobacillus acidophilus.*

Leben, consumed in Egypt, contains rather more alcohol than the preceding beverages. **Koumiss,** made in Russia from mares' milk, also contains lactose-fermenting yeasts as well as lactic bacteria, and may have an alcohol content of 3 per cent. **Kefir,** popular in the Caucasus, is made by the symbiotic action of *L. caucasicus,* yeasts, and other bacteria. Kefir and the Norwegian **kaeldermilk** seldom contain more than 0·5 per cent. alcohol and 1·5 to 2·5 per cent. lactic acid.

For further details of fermented milk drinks reference may be made to Orla-Jensen (1931) and to Palladina *et al.* (1935).

Other Alcoholic Beverages.

Many fermented drinks are made from fruit juices other than grape, notably **cider** from apples. The general principles of control are similar to those for wine making. A good book on cider is that of Warcollier (1928).

Certain other sugary juices provide a source of fermentable material. Indian **toddy** is made from the juice of the toddy

palm, and Mexican **pulque** from the juice of the agave, in both cases by the action of a mixed culture of spontaneously developing yeasts and bacteria.

The Japanese alcoholic beverage **saké** is prepared from rice, the preliminary saccharification being effected by *Aspergillus oryzæ*. Subsequent fermentation is by yeasts, and the alcohol content may reach 20 per cent. or even more. South African **kaffir beer** is a thin fermented mash of millet.

The **ginger beer plant** used as an inoculant for the fermentation of ginger beer consists of a yeast acting in symbiosis with a bacterium. The "Palestine Bees," popular some years ago, also consist of yeast plus a lactic bacterium—possibly the same species. The lumps of yeast rise to the surface as they collect gas, give off the gas and sink, only to rise again as further gas collects.

Soy Sauce.

Of the fermented food products popular in the Far East, soybean sauce is the most important. A damp mixture of soybean and wheat flour is inoculated with *Asp. oryzæ* or related fungus, brine is added, and the mash then subjected to a very prolonged fermentation, in which bacteria and yeasts also take part.

Vinegar.

Vinegar contains 4 to 8 per cent. of acetic acid, and is made by the alcoholic fermentation of sugary or starchy material, followed by an acetic fermentation of the alcohol. The raw material may be wine (vinegar=vin aigre), sour beer, fruit juices, molasses, or grain. In Britain vinegar is usually prepared from malted barley, and the first part of the process is very similar to the preparation of beer.

The fermented wort (which is unhopped) is usually stored for some time to allow the yeast cells to deposit, and is then run into large wooden acetifiers, filled with some inert material over which the alcoholic solution trickles. The object is to provide as large a surface as possible, and to ensure that the acetic fermentation takes place under aerobic conditions. The filling material is usually beech shavings or birch twigs, but many other substances—such as basket work, wood char-

coal, fabrics, and maize cobs—have been employed. The alcoholic liquor, containing about 6 per cent. alcohol, which gives about 5 per cent. acetic acid, is mixed with about one-quarter of its volume of good vinegar, and run in gradually over the filling material. A grid at the bottom of the acetifier keeps a clear space from which the liquid may be drawn off as required. Air is admitted through holes in the side of the vessel just below the bottom grid. The filling material gradually develops a film of acetic bacteria, and the alcoholic solution is continuously passed over this until the alcohol is almost all converted to acetic acid. The acetifier, when in use, should not be stopped for more than a day or two at a time, or mould fungi and other infections may develop. It may be run for six to nine months, after which time it should be cleaned and started afresh.

The temperature of the acetifier should be 35° to 40° C., and once started the oxidation of the alcohol maintains the necessary heat.

After-treatment of the vinegar includes storage, and filtration or clarification. For further information see Mitchell (1926).

Vinegar Bacteria.—The film of acetic organisms, surrounded by mucilaginous sheaths, which forms on the surface of wine exposed to the air, was termed by Pasteur *Mycoderma aceti*. This name is no longer used, for it has been found that there are a number of distinct species of bacteria capable of developing such a film. All are aerobic, and the best known species are *Acetobacter aceti*, *A. pasteurianum*, and *A. kützingianum*.

Distilled and Industrial Alcohol.

Beverages of high alcoholic content are prepared by distilling the yeast-fermented extract obtained from raw starchy and sugary materials, the distillate being subsequently diluted to give a standard alcoholic content. Special strains of yeast (distiller's yeasts) giving a high alcoholic yield are usually employed.

Starchy materials are first converted to sugars by malting, as in brewing; by diastatic moulds—*e.g.*, Boulard process—or by acid hydrolysis. **Whisky** is prepared from a wort made from malted barley or other cereals, or from potatoes, and

contains 40 per cent. alcohol. In **gin** the distilled spirit (obtained mainly from maize) is flavoured with juniper berries, together with other ingredients such as coriander, cinnamon, and angelica root.

Saccharine substances such as molasses or grapes are generally treated as described for wine manufacture. **Rum** is distilled from fermented cane sugar molasses, and **brandy** from grape wine.

Alcohol for power or other industrial purposes is now largely obtained by the yeast fermentation of cane or beet sugar molasses. Owing to the heavy duty on alcohol in most countries, the economics of alcohol production is often obscure. In India, where cane sugar molasses is at present almost a waste product, the production of alcohol is said to be impracticable; yet large quantities of molasses are brought to England from the West Indies, and fermented by commercial enterprise.

Power alcohol for internal combustion engines is a rather attractive proposition. It is one of the few sources of motive power that will be independent of the coal and petrol famine that is bound to come sooner or later. At present it can only compete with petrol under exceptional conditions (see D.S.I.R., 1927).

In distilling fermented liquors, the alcohol is concentrated to about 95 per cent. strength, and the "head products" (acetaldehyde and volatile esters) and "tail products" (fusel oils) are removed.

Glycerol.

The production of glycerol by fermentation was exploited in Germany as a war-time measure (Connstein and Lüdecke, 1919). When molasses or other sugary liquor is fermented—*e.g.*, by *Sacch. ellipsoideus*—the addition of 5 per cent. of sodium carbonate makes the medium alkaline, and the yield of glycerol is increased. The addition of sodium sulphite produces a similar effect, and has the advantage of suppressing undesirable bacteria. A glycerol yield of 36 per cent. has been recorded when twice as much sulphite as sugar is used. The yeast can be regenerated by a purifying fermentation in the presence of dilute acid, but very high concentrations of sodium sulphite permanently injure the yeast. In 1919 the German

output by this method was 1,000 tons per month, the average yield being 20 to 25 per cent. on the sugar used; in addition, large quantities of alcohol and acetaldehyde were produced as by-products.

Production of Organic Acids and Industrial Solvents.

The production of acetic, butyric, lactic, propionic and gluconic acids by bacteria, and of citric and gluconic acids by fungi, is capable of economic exploitation. A summary of the literature of these fermentations is given by Thaysen and Galloway (1930), Buchanan and Fulmer (1928-30), and Wells and Ward (1939).

Acetic Acid fermentation has already been considered under the heading of Vinegar; it is again referred to below under the heading of Cellulose Fermentation.

Butyric Acid, Butyl Alcohol and Acetone.—Economic interest in the butyric bacteria, and particularly those strains that convert a considerable part of the acid into butyl alcohol, started shortly before 1914, in connection with attempts to manufacture synthetic rubber. The abnormal war-time demands for a source of acetone (for cordite manufacture) within the country led to a development of the process for the sake of the acetone which is also produced by this fermentation. Butyl alcohol—in quantity double the acetone—became almost a waste product, a certain amount being used for aeroplane "dopes," etc. Later on, the introduction of cellulose lacquers led to the increased use of butyl alcohol for solvent purposes. The fermentation process was in consequence still run successfully on the other side of the Atlantic, both butyl alcohol and acetone finding a market. Recently the process has been restarted in England.

An account of the butyl alcohol-acetone fermentation is given by Thaysen (1921). A starchy mash—*e.g.*, maize—is inoculated with a pure culture of *Clostridium butyricum* (*Cl. acetobutylicum*), and subsequent fermentation at 37° C. is rapid, the process being complete in about thirty-six hours. A yield of 26 per cent. on the weight of the maize is obtained: butyl alcohol, acetone, and ethyl alcohol are produced in the approximate ratio 6:3:1, and are separated by fractional distillation. The fermentation is extremely susceptible to

infections, and for successful results it is essential that the mash should be adequately sterilised. Xylose may be used to replace part of the maize mash (Underkofler *et al.*, 1936).

Ethyl Alcohol-Acetone Fermentation.—Acetone is also produced, in addition to a larger quantity of ethyl alcohol, by *Bac. acetoethylicus* acting on starch and sugars. This organism possesses large, terminal, oval spores, and under certain conditions produces characteristic volutin granules. It is of interest as being one of the few organisms (the butyl alcohol-acetone organism is another) capable of fermenting pentoses, which may also be used to yield an ethyl alcohol-acetone mixture amounting to 33 per cent. of the pentose consumed. The acetone varies from 5 to 10 per cent. of the mixture, and adds to its value as a motor fuel (Northrop *et al.*, 1919).

Thaysen and Galloway (1928) carried out experiments with this organism on a semi-technical scale to investigate the value of various raw materials as a source of motor fuel. They found that yields of the order of 20 gallons per ton of raw material could be obtained from rice straw, tropical grasses, and maize cobs. Hydrolysis involving both hot acids and pressure treatments presented technical difficulties, and it was found better to conduct the conversion with stronger acid and at atmospheric pressure. It is doubtful whether such a process could be economically worked at the present time, but it provides a possible means of producing power alcohol from a source that is quite unlimited and of no value as a food material.

Citric Acid.—Citric acid is produced by the action of certain moulds on sugar solutions. Despite the increase in citrus cultivation during recent years, the fermentation product is capable of competing with the fruit product, and is commercially manufactured in Britain.

Citric acid formation was first observed with certain monoverticillate strains of *Penicillium*. Better results are given by strains of *Aspergillus niger*, which has the advantage of being able to withstand an acid reaction sufficient to suppress most infections. Various workers record yields of 90 per cent. and more on the weight of saccharose or dextrose fermented. Recent summaries on this subject are those of Doelger and Prescott (1934), and Wells and Herrick (1938).

Lactic Acid.—Lactic acid finds industrial use in the woollen

and leather industries. It may be prepared by bacterial fermentation of waste milk products, or from molasses and other sugary materials. The latter type of raw material is to be preferred, since it makes the removal of the lactic acid a simpler matter. Lime is added during the fermentation to prevent the reaction becoming too acid, and when all the sugar has disappeared the mash is filtered, concentrated, and treated with sulphuric acid to liberate the lactic acid and to precipitate the calcium.

A more recent process utilises the mould *Rhizopus oryzæ*. A 13 per cent. dextrose solution at 35° C., and an excess pressure of 5 lb. to the square inch, is fermented in thirty to thirty-six hours, giving a yield of lactic acid amounting to 70 to 75 per cent. of the dextrose. This compares well with the bacterial process, and a special feature is that whereas the latter gives a mixture of *d*- and *l*- acids, the mould produces *d*- lactic acid entirely.

Gluconic Acid.—The formation of gluconic acid by certain acetic bacteria was demonstrated over fifty years ago. More recently it has been shown that certain species of *Aspergillus* and *Penicillium* will also form gluconic acid, when acting under aerobic conditions and at a reaction near to neutrality.

A crude gluconic fermentation is carried out in the preparation of the fermented tea drink *kombucha* in China, where sweetened tea is fermented by acetic bacteria. The commercial exploitation of gluconic acid fermentation seems to be a possibility, but no figures are available as to the relative costs of biological and chemical methods of manufacture. Calcium gluconate is of value as a readily assimilable remedy for calcium deficiency.

Propionic Acid.—This is produced by an anaerobic decomposition of hexose sugars, in which lactic acid is an intermediate product. Some types of propionic bacteria are associated with the formation of the characteristic gas holes in Emmenthal cheese. Various economic uses for propionic acid have been suggested, and fermentation methods worked out for its industrial preparation. A good account of the propionic acid organisms is given by van Niel (1928).

Methane Formation and Cellulose Fermentation.

The fermentation of cellulose by thermophilic bacteria producing methane as a by-product was first studied thirty years ago by Omelianski (see Thaysen and Bunker, 1927). Ten years before this cellulose waste had been used—on an experimental scale—both in England and in India, as a source of light and power. More recently thermophilic fermentation has been used to produce methane in large quantities for research on mine explosions.

A process has been worked out for fermentation of cellulosic material such as maize cobs, beet pulp, etc., to give acetic acid as the main product. Small additions of ammonium salts and phosphates are added to the mash, which is maintained at 60° C. and at a neutral reaction. A crude inoculant of horse dung is used, the high temperature restricting bacterial development to the types required. In addition to acetic acid, smaller quantities of butyric acid, alcohol, methane and carbon dioxide are produced (Langwell, 1932). Veldhuis, Christiansen and Fulmer (1936) have studied the thermophilic mixed culture fermentation of cellulose with particular regard to increasing the proportion of ethyl alcohol formed. At 55° C. and pH 7·5 the cellulose was practically all consumed in ten to twelve days, giving 26 per cent. alcohol and 24 per cent. acetic acid.

Bread-making.

Bread-making may logically be considered as a fermentation industry, but it differs from the alcoholic fermentations already described in that the alcohol is merely a by-product. The primary purpose served by the yeast is the production of a gas (carbon dioxide) that distends the glutinous flour paste into a spongy mass, thus making the baked product light and palatable. Such aeration can be produced by purely chemical or mechanical means—from "baking powder" or from gas cylinders, for example—but the yeast process as usually employed is more convenient and has certain advantages, amongst which may be mentioned the favourable effect of the yeast enzymes on the flour proteins, and possibly the food value of the yeast itself.

For baking purposes a distillers' yeast, usually a top fermenta-

tion type, is preferable to a brewers' yeast; it is more active in gas production and grows better in flour pastes. Compressed yeasts used for bread-making frequently contain a proportion of lactic bacteria, and a mild lactic fermentation adds to the flavour of the bread. The development of butyric bacteria, on the other hand, imparts a sour taste. French bread is not usually made with compressed yeast, but with a " leaven " or " sour dough " starter containing lactic and butyric bacteria in addition to yeast. " Salt rising " bread, as made in certain districts of America, employs a starter which is made by adding hot milk and salt to corn meal; spore-forming bacteria survive this treatment and provide the necessary gas production.

Where compressed yeast is responsible for the " rising " of bread, the presence of fermentable sugars is, of course, implied. Flour after milling contains 1 to 2 per cent. saccharose and a trace of dextrose. The addition of a little malted flour increases the sugar content by converting some of the starch to maltose. It is desirable that throughout the rising process the production of sugar should roughly keep pace with the conversion of the existing sugar into alcohol and carbon dioxide, in order that an even rate of gas production may be maintained, and that a small residue of sugar should remain to improve the flavour of the bread, and to assist in the formation of a brown crust. The development of the yeast is assisted by the addition of " improvers," such as phosphates or ammonium salts.

Defects caused by Micro-organisms.—The temperatures reached by the loaf during baking are approximately 140° C. at the surface and 100° C. at the interior. Certain bacterial spores can survive the lower temperature, notably those of the *Bac. mesentericus* group, which if numerous lead to " ropiness " in the stored bread. Remedial measures include the use of flour that is less infected, a more acid reaction of the dough, rapid cooling after baking and cool, dry storage. The souring of bread by butyric organisms in the leaven has already been mentioned; similar souring may occur in stored bread as a result of bacterial spores having survived the baking process.

Serratia marcescens (*Bact. prodigiosum*), which is capable of causing a blood-red surface growth on bread, is the origin of the appearance of the " bleeding Host." Orange and red

growths have also been traced to certain fungi, such as *Monilia sitophila*. Under damp storage conditions bread rapidly develops mould growths of the *Penicillium* type. A technique for estimating the " rope " spore content of flour is given by Amos and Kent-Jones (1931).

Commercial Production of Yeast.

Yeast was formerly a by-product of the brewing and distilling industries. The increased demand for pure yeast for bread-making and other purposes has led to its manufacture as a main product. Fermentation is conducted in the presence of an ample air supply in order to obtain the maximum yield of yeast. The necessary carbohydrate is supplied in the form of molasses or other cheap sugary liquor, and ammonium salts may be added as an additional source of nitrogen.

Special yeasts may be cultivated for purposes other than their fermenting power. *Torula utilis*, the so-called " mineral yeast," has been grown on a large scale as a means of converting inorganic into organic nitrogen, and *Endomyces vernalis* as a means of converting sugars into fats.

Yeast is finding an increasing number of uses in food and medicine. It contains vitamin B and ergosterol, and is often recommended for certain blood complaints. Ergosterol, which is a source of vitamin D, is also obtained from several moulds. A vegetarian " meat extract " from yeast is very palatable. Commercial invertase preparations from yeast are used in the confectionery industry. Yeast may be preserved during transport by cold storage, freezing or drying.

A summary of recent advances in the fermentation industries is given by Chrzaszcz and Janicki (1936), whilst the fermentative aspects of fungi are described by Ramsbottom (1936).

REFERENCES

ALLEN, P. W. (1926): Industrial Fermentations. New York.
AMOS, A. J., AND KENT-JONES, D. W. (1931): Analyst, 56, 572.
BUCHANAN, R. E., AND FULMER, E. I. (1928-30): Physiology and Biochemistry of Bacteria. London.
CHRZASZCZ, T., AND JANICKI, J. (1936): Chem. and Ind., 55, 884.
CONNSTEIN, W., AND LÜDECKE, K. (1919): Ber., 52, 1835.
CRUESS, W. V. (1934): Principles and Practice of Wine Making. New York.

DEPT. SCI. IND. RESEARCH (1927): Fuel for Motor Transport: Fourth Mem. H.M. Stationery Office.
DOELGER, W. P., AND PRESCOTT, S. C. (1934): Ind. Eng. Chem., **26**, 1142.
HOPKINS, R. H., AND KRAUSE, B. (1937): Biochemistry Applied to Malting and Brewing. Allen and Unwin.
LANGWELL, H. (1932): Chem. and Industry, **51**, 988.
LAUFER, S., AND SCHWARZ, R. (1936): Yeast Fermentation and Pure Culture Systems. New York.
LUERS, H. (1929): Chemie des Brauwesens. Berlin.
MITCHELL, C. A. (1926): Vinegar, Its Manufacture and Examination. London.
NORTHROP, J. H., ASHE, L. H., AND SENIOR, J. K. (1919): J. Biol. Chem., **39**, 1.
ORLA-JENSEN, A. D. (1931): Dairy Bacteriology. London.
PALLADINA, O. K., *et al.* (1935): Milchw. Forsch., **17**, 222.
RAMSBOTTOM, J. (1936): Chem. and Industry, **55**, 717.
SHIMWELL, J. L. (1935-36): J. Inst. Brewing, **41**, 245, 481; **42**, 119.
—— AND KIRKPATRICK, W. F. (1939): J. Inst. Brewing, **45**, 137.
SMYTH, H. F., AND OBOLD, W. F. (1930): Industrial Microbiology. Baltimore.
THAYSEN, A. C. (1921): J. Inst. Brewing, **27**, 529.
—— AND BUNKER, H. J. (1927): Microbiology of Celluloses, etc. London.
THAYSEN, A. C., AND GALLOWAY, L. D. (1928): Ann. Appl. Biol., **15**, 392.
—— —— (1930): Microbiology of Starch and Sugars. London.
UNDERKOFLER, L. A., CHRISTENSEN, L. M., AND FULMER, E. I. (1936): Ind. Eng. Chem., **28**, 350.
VAN NIEL, C. B. (1928): The Propionic Acid Bacteria. Haarlem.
VELDHUIS, M. K., CHRISTENSEN, L. M., AND FULMER, E. I. (1936): Ind. Eng. Chem., **28**, 430.
VISSER 'T HOOFT, F., AND DE LEEUW, F. J. G. (1935): Cereal Chem., **12**, 213.
WALKER, T. K. (1930): Chem. and Industry, 946.
—— (1931): J. Inst. Brewing, **37**, 512.
—— (1935): *Ibid.*, **41**, 92 and 472.
WARCOLLIER, G. (1928): Le Cidrerie. Paris.
WELLS, P. A., AND HERRICK, H. T. (1938): Ind. Eng. Chem., **30**, 255.
—— AND WARD, G. E. (1939): *Ibid.*, **31**, 172.

CHAPTER XI

TEXTILE INDUSTRIES

Retting.

VEGETABLE "fibres" used for textiles may be seed hairs, leaf fibres, or stem fibres. The last class includes the important raw materials flax, jute and hemp, whose fibres are separated from the stems by the process known as *retting*.

The essential feature of retting is the removal of the pectic substances surrounding the fibre-bundles by pectinase—an enzyme secreted by many bacteria and fungi—thus liberating the bundles from the surrounding tissues. For this purpose a crude culture of suitable organisms is obtained, usually by steeping the plant stems in water under suitable conditions.

Various methods of retting flax are employed, some anaerobic and some aerobic. In the former, the plant stems are tied in bundles and are submerged in slow-running streams (Germany, Holland and Belgium) or stagnant water (Ireland, Italy). The organic substances diffusing into the water act as nutriment for many aerobic bacteria and fungi, whose growth results in the enhancement of anaerobic conditions; this in turn favours the development of anaerobic pectin-decomposing bacteria, such as *Clostridium* spp. After the retting process the material is mechanically treated—*i.e.*, scutched and combed—to eliminate the woody and soft tissues, leaving the bundles of fibre tissue for cleaning, preparing and manufacturing into yarns. The period of retting depends upon the conditions, including temperature. At 20° C. it is about 100 hours. Frothing occurs owing to the evolution of carbon dioxide and hydrogen, and organic acids (acetic, lactic and butyric) together with alcohol, acetone and nitrogenous substances are produced. During subsequent air drying, aerobic bacteria may oxidise the organic acids and hence assist in cleaning. Over-retting, which occurs if prolonged exposure to the retting water is given, results in

undesirable changes. Loss of strength due to cellulose decomposition may occur, and attack on the pectin may go too far, so that not merely the fibre bundles are liberated, but also the individual or " ultimate " fibres. Careful control of the retting process is therefore important, and can to some extent be effected by following the development of acidity (Eyre and Nodder, 1924).

Desmedt (1933), who reviews the literature on flax retting, concludes that the most suitable retting waters are soft, warm, slow flowing, and free from iron, which is apt to colour the fibre.

Many modifications have been introduced into the retting process. One is to speed up the process by raising the temperature of the water. Thus with a warm water ret at 37° C. the process may be completed in forty to fifty hours, but very careful control is necessary to avoid over-retting. Another modification involves continuous or periodical changing of the water and blowing through a moderate current of warm air; this checks the development of acidity, and is stated to result in an improved fibre.

Various patents have been taken out for the use of pure cultures, such as *Clostridium butyricum* (*Bac. amylobacter*) and *Cl. felsineum*. Entirely aerobic processes have been recommended, and have the advantage that they do away with the unpleasant-smelling waste liquors whose disposal presents such a problem. In Rossi's process (1916) the liquor is kept at 28°-30° C., inoculated with a starter culture of the aerobic spore-forming *Bac. comesii*, and air forced through. Anaerobic forms are not entirely suppressed. The retting of hemp and flax is completed in about two days. The spent liquor is almost odourless, has little acidity and can be disposed of without difficulty. The risk of over-retting is considerably reduced.

Another aerobic process that has long been in use is **Dew Retting.** The fibre plants are spread in thin layers on the natural vegetation—*e.g.*, heathland and moorland. They are kept damp by rain and dew and various fungi, chiefly *Cladosporium herbarum* and perhaps *Mucor hiemalis* and *Rhizopus nigricans*, develop on them. The fungal mycelium penetrates the plant stems and provides the retting enzymes. Aerobic bacteria may also participate. Dew retting takes about seven days in summer and considerably longer in winter, when it is also more irregular.

Retting of less valuable fibres—*e.g.*, jute—is carried out on similar principles, but usually with less care. The retting of ramie presents peculiar difficulties, and physical or chemical methods are usually adopted.

A fuller account of the principles of retting is given by Thaysen and Bunker (1927).

Mildew and Decay of Textiles.

Considerable damage may be done at nearly all stages of textile manufacture by fungi and bacteria. Such damage has been most fully investigated in connection with the two main textiles, cotton and wool. Two types of attack may be distinguished—"*mildew*," leading to a musty smell and discoloration of the material, and *decay* or rotting, leading to loss of strength. There is, of course, no sharp dividing line between these two forms of attack.

Mildew is usually caused by the common saprophytic moulds, notably species of *Aspergillus* and *Penicillium*, whose spores come from the atmosphere, or may be introduced with the raw material. Since infection cannot be avoided, control measures are best directed to the following three points:

(i.) Maintaining the material at a safe moisture content.

(ii.) Elimination of substances capable of acting as food for micro-organisms.

(iii.) Incorporation of antiseptics.

Any problem relating to mildew or decay of textiles may usually be solved by suitable attention to one or more of these points. It should be borne in mind that low liability to mildew may be associated with either (ii.) or (iii.) above, and the terms *mildew capacity* and *mildew resistance* have been coined to express this distinction.

Decay of textiles may be caused by either mould fungi or bacteria, generally by both combined. When there is free access of air and the material is not too damp, as in tents and awnings, fungi predominate; under very wet conditions—*e.g.*, fishing nets—bacteria predominate.

Cellulose fabrics, such as curtains, blinds, tents and awnings, may deteriorate through the action of chemicals or light, as well as by the action of micro-organisms. Searle (1929), who describes a useful method for comparing the resistance of

cloths to decay, was the first to point out that biological decay of cellulosic materials can readily be distinguished from light decay by the fact that the former has little or no effect on the viscosity in cuprammonium of the affected cellulose.

For the prevention of decay in such fabrics soluble antiseptics are of little use except during the early exposure to damp conditions. This is often sufficient, since the material becomes less liable to decay after the soluble substances have been washed out by rain. For more permanent protection it is necessary that the fabric should be impregnated with a proofing substance, either by simple waterproofing, by tarring, or treatment with some such substance as copper oleate. Copper and zinc naphthenates afford good rot-proofing, and the latter has the advantage of being colourless. Neill and Travers (1938) recommend for the protection of tent calico either an iron-chromium treatment or immersion in 1 per cent. salicylanilide.

The diagnosis of mildew or decay due to micro-organisms is not always easy. A musty smell is usually the first indication. Then the presence of the organisms in adequate quantity must be shown, preferably in comparison with a sample of unaffected material. Fungal mycelium (and spores if present) is best looked for by mounting a teased-out specimen in lactophenol-cotton blue (see Chapter V.). Bacteria may be looked for in preparations stained with carbol fuchsin. It may be noted here that the lactophenol-cotton blue mounting medium is particularly useful for textile work. The cotton blue stains fungal mycelium, but not cellulose fibres; real silk takes the stain, whereas rayon does not; and damage to wool and other hairs is shown by an increased capacity to take the stain (Nopitsch, 1933).

If the damage is of recent occurrence, it may be possible to obtain cultures of organisms present by suitable technique, such as scattering finely chopped portions of the material on the surface of an agar plate. Since, however, even sound material usually contains numerous micro-organisms, extreme discretion is necessary in interpreting results obtained by this means.

Chemical methods for indicating mildew and decay are also available, their nature depending on the textile material under

study. The swelling test (Fleming and Thaysen, 1921) and the Congo red test (Bright, 1926) are particularly useful in showing structural damage to cotton, while the Pauly test is suitable for wool (Burgess and Rimington, 1929). The use of trypsin preparations in determining the susceptibility of wools to bacterial attack will be referred to later.

Cotton.

The mildew problem is particularly important for cotton, since the raw material and " grey " (unbleached) yarn and cloth made from it contain nitrogenous food material and act as an excellent substratum for the growth of micro-organisms. Mildew in bleached and finished goods is also not uncommon, the nutrient in this case being derived from the finishing material employed—*e.g.*, dextrin, glycerol and soluble oil.

Infection takes place from the cotton field onwards, and only requires sufficiently moist conditions in order to develop and cause damage. Unfortunately, the cotton industry—for many reasons, of which some are sound and some not—aims at producing moist conditions at almost every stage of manufacture. As a result all cotton goods are mildewed to some extent, however slight, and the question as to whether a particular sample is or is not mildewed has frequently a technical rather than a scientific basis, depending on the keenness of the observer's nose and eyes, together with the state of trade.

An account of the commonest types of fungi occurring on cotton has been given by Bright, Morris and Summers (1924) and by Galloway (1930). Species of *Aspergillus* and *Penicillium* predominate, the former being favoured by warm storage conditions. Coloured stains are a frequent source of complaint, and may be due either to spore masses (*e.g.*, of *Aspergillus niger*) or more commonly to pigment formation either within the hyphæ (as in the greenish-black mycelium of *Cladosporium*) or to soluble pigments produced (as in the red or purple stain caused by *Fusarium*). Yellow or orange stains due to coloured perithecia of the *Aspergillus glaucus* group are also not uncommon on yarn or cloth stored under conditions only just moist enough to permit mildew development.

Many of the fungi produce acid from starch, and such types are important in the case of certain dyed goods, where the dye

may act as an indicator and change in colour wherever the slightest amount of mildew has developed on the starch or dextrin filling. The amount of fungal mycelium in such cases may be so small that it can be detected only by careful microscopic examination.

On raw cotton materials, slight alkalinity is a usual consequence of fungal and bacterial growth, but may be masked subsequently by acid production from sizing or softening ingredients—*e.g.*, starch, glycerol.

Ability to decompose cellulose is possessed, in varying degree, by about half the commonly occurring fungi; among the more active fungi capable of causing " tendering " or loss of strength are *Aspergillus fumigatus*, *Cladosporium herbarum*, and various species of *Stemphylium*, *Chætomium* and *Penicillium*.

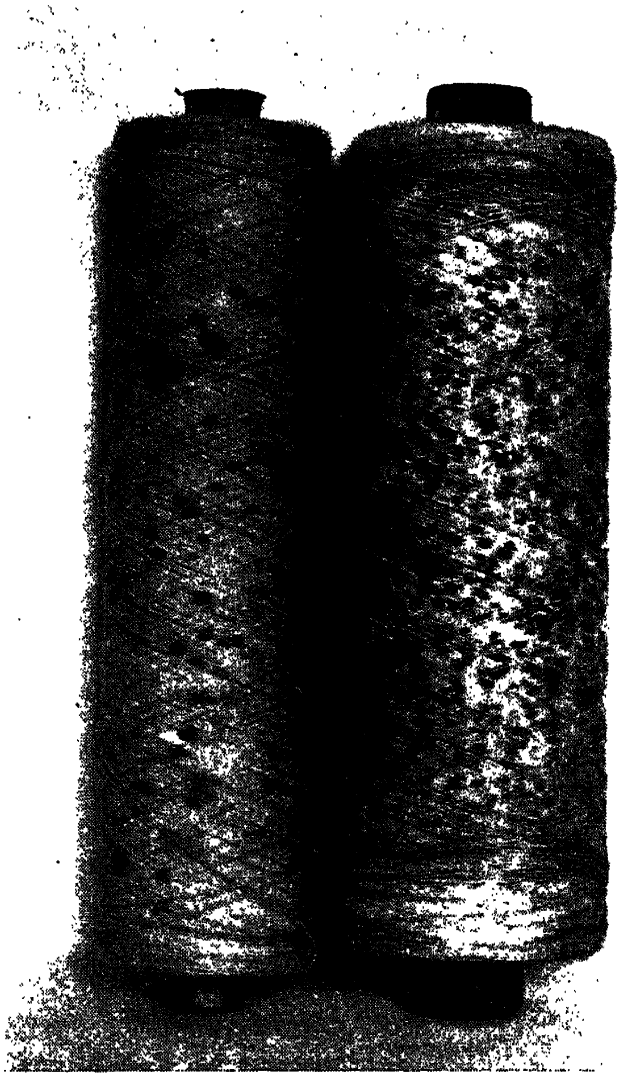
Attack by fungi has been considered first, since, owing to the lower moisture requirements of the fungi, this is far more common than bacterial attack. The safe limit for storage of cotton is a moisture content of about 8 per cent. (equal to a " regain " of 9 per cent.),* or a storage atmosphere of 70 to 75 per cent. relative humidity. At relative humidities approaching 100 per cent. the development of bacteria is rapid, within the lumen of the cotton hair as well as on the surface. Such bacteria are usually harmless, or at the most produce a slight smell or discoloration; but if exposure to damp conditions is prolonged, active cellulose decomposers—*e.g.*, *Cytophaga hutchinsoni*—may cause weakening of the fibre.

Stages at which Micro-organisms Develop.—Micro-organisms may develop at all stages during the manufacture of cotton goods.

In the Field.—Even before the boll opens, the cotton may be attacked by *Aspergillus niger* or *Rhizopus nigricans*. Puncture by the " stainer " bug frequently introduces a yeast-like fungus *Nematospora*, which is responsible for the brown stains so common in many types of cotton, and also leads to immaturity of the cotton hairs.

In the Bale.—Fungi and bacteria are frequently a cause of stains or tendering in bales that have been allowed to become damp. In this connection the risk of the common practice of watering cotton at the time of baling becomes obvious.

* *Moisture regain* is calculated on dry weight, *moisture content* on wet weight, unless otherwise stated.



BOTANY YARN MILDEWED AS THE RESULT OF OVER-CONDITIONING.

[*Facing page* 132

In Yarn.—Yarn frequently mildews owing to damp storage conditions. Many of the methods employed in " conditioning " yarn—*i.e.*, increasing its moisture content—are still of a crude nature, and frequently lead to local excess of moisture and consequent development of fungi and bacteria. It is a common practice to put formalin or some other suitable antiseptic in the water used for " conditioning " or for " wet doubling." The yarn to be used for the " warp," across which the shuttle containing the " weft " passes during weaving, is " sized " with a starch paste in order to enable the threads to resist abrasion. Imperfect drying of the sized warp is a common cause of mildewing.

In Cloth.—Weaving sheds are usually humidified, and mildew often occurs in warp and cloth on the looms. This is especially so after a few days' stoppage, when the temperature has dropped and condensation of moisture has occurred. Outbreaks of " weaver's cough " appear to be definitely related to mildewing and the resulting distribution of spores in the atmosphere. Unbleached cloth is frequently stored in damp warehouses, or exported to the East and exposed to monsoon conditions, and is found to develop coloured stains and a musty smell. Most striking colours and patterns—the latter sometimes diamond-shaped owing to the more rapid growth of the mildew in the directions of warp and weft (Galloway, 1931)—are often met with.

To give some protection against border-line storage conditions (absolute protection is difficult, and not worth the extra expense), it is customary to add an antiseptic to the cloth. Zinc chloride is the antiseptic usually employed, but newer substances, such as salicylanilide, are coming into use. The many requirements of a good textile antiseptic are discussed by Fargher, Galloway and Probert (1930). The antiseptic is applied to the cloth by being added to the size mixture, and this procedure, although originally based on a mistaken belief that the size was the sole nutrient for the mildew, is a perfectly satisfactory method in practice.

In " finished " cloths, the nutrient material of the cotton has been removed by " scouring " and bleaching, and any fungal growth must be nourished by the filling material (usually dextrin paste) or softeners used. In dyed materials the nature of the dye is of importance; it may be sensitive to acid produc-

tion, it may act as a nutrient for mildew, or it may act as an antiseptic and check mildewing.

Use of Fermented Flour as Sizing Material.—When wheat flour is used for sizing in the cotton industry, the common practice is to give it a preliminary " fermentation " in order to decrease its viscosity. A 50 per cent. flour-water paste is made up in a large wooden vat provided with a stirrer, and kept at ordinary air temperatures, usually 15°-20° C. During the first few days a varied micro-flora develops, including yeasts which ferment the small amount of sugar present in the flour and lead to very considerable frothing. After a week this has died down, and the predominating organism—which by virtue of the acidity it produces suppresses most of the other organisms originally present—is a particular type of lactic acid bacterium. The fermentation is kept going for weeks or even months, and produces desirable changes in the sizing qualities of the paste. Provided that stirring is thorough the process is a very constant one, but if stirring is insufficient anaerobic butyric organisms develop and give the size, and hence the cloth, an objectionable odour.

Wool.

Wool and other hair fibres differ from the vegetable fibres in being essentially protein and not cellulosic in nature. Under ordinary conditions they are probably less susceptible than vegetable fibres to fungal attack, but are more susceptible to certain bacteria.

The main factors determining microbiological growth are food and moisture. While the chemically clean fibre is very resistant, the presence of foreign matter—*e.g.*, natural grease and suint, added soaps and oils, products of alkali hydrolysis or body secretions—provides foods for micro-organisms. Bacteria develop and cause discoloration and deterioration when the wool is wet and exposed to air, as on the sheep's back in rainy weather, or during an interval between wet processing. The organisms belong to the *Bacillaceæ*—*e.g.*, *Bac. subtilis* and *Bac. mesentericus* are particularly active. *Pseudomonas pyocyaneus* may discolour wool green while on the sheep, and also tender it. Anaerobic bacterial decomposition probably does not occur; large quantities of wool submerged for thirteen years

in 300 feet of water have been found to be in good condition. Owing to the hygroscopicity of wool, it has been found necessary to adopt a standard moisture regain—viz., about 16 per cent.—on the dry weight as a basis for trade. Furthermore, a certain amount of moisture must be present to prevent static electrical effects which interfere with its manipulation. The application of excess moisture during the so-called conditioning process may lead to mildew during subsequent storage. The structural condition of the fibre is also important, previously damaged fibres being more susceptible to attack. The number of species of mould fungi causing trouble in ordinary practice is less than in the case of cotton, the commonest genera being *Penicillium* and *Aspergillus*, and to a less extent certain strongly proteolytic fungi imperfecti and mucors. On a commercially clean botany wool, moulds develop at a moisture " regain " of about 24 per cent., corresponding to a storage humidity of about 94 per cent. With less clean wool the critical humidity may be lower. Bacteria and actinomycetes require more moisture; perhaps a condition of actual wetness. Some actinomycetes effect a rapid disintegration of the fibre, and certain species may do this under thermophilic conditions. The most frequent type of trouble in wool is discoloration with consequent irregularity in dyeing properties. Fibre deterioration follows the surface establishment of the fungus, which occurs at the expense of foreign soluble matter. The greater the amount of water-soluble nitrogenous matter, the greater the mildew liability.

Fibres of wool and hair are composed of strongly keratinised animal cells, presumably cemented together by some protein substance less resistant to proteolytic enzymes. Structural deterioration of the fibre is initiated by a loosening of the scales of the protective epithelium, this being followed by the separation of the spindle-shaped cortical cells. Burgess (1934*a*) has shown the reaction to resemble that produced by trypsin. The individual cortical cells do not appear to decompose, although there is reason to believe that in specially favourable circumstances for decay—*e.g.*, in a loamy soil—they do so. The pH of the fibre is also of importance, for, whereas alkaline conditions allow and often stimulate both fungal and bacterial growth, acidity checks bacterial action. Contact with the more mildew-susceptible vegetable materials

e.g., paper cones, cotton yarns in union goods, etc.—sometimes leads to trouble with wool, the main defect being one of discoloration.

In the mill, control of mildew can be effected by keeping the moisture content as low as possible consistent with trade practice. The humidity of storage rooms for tops and cheeses of yarn, etc., must be kept constant throughout the rooms (as indicated in Chapter VIII.) and held below 85 per cent. The standard regain of 16¼ per cent. corresponds to a relative humidity of about 70 per cent. When wool passes out of the manufacturer's hands, as during transhipment of yarn abroad, it may be expedient to apply antiseptics before despatch. Such antiseptics should be innocuous to humans, should not affect the desirable properties of the goods, and should be easily removed during an ordinary scouring process. The treatment is carried out preferably by immersing the wool in a weak bath—*e.g.*, during backwashing—followed by drying. Application of antiseptics during any of the conditioning processes is not successful, except, perhaps, in the case of clean, high quality merino (botany) wool. Suitable antiseptics are sodium salicylanilide and sodium silicofluoride, applied in a concentration of 0·1 per cent. on the weight of the goods. Certain moth-proofing compounds also exercise a protective effect, as do the ordinary processes of chrome-dyeing. Where goods are likely to remain wet for some time—*e.g.*, during cuttling between processes—bacterial action is inhibited by previously immersing them in a weak bath of acid or sodium silicofluoride. Failing this, it is better to immerse them completely in a deep bath of water. In certain cases it is possible to treat the fibre chemically in such a way as to bind the cortical cells more firmly together, thereby giving protection against the harmful bacterial enzymes. By using a 0·25 per cent. solution of trypsin, buffered to pH 8·6 and incubating at 37° C., the effect of such treatments can readily be ascertained (Burgess, 1934*a*). Thus it is possible to determine within twenty-four hours, or in the case of a very susceptible sample of wool within a few hours only, the resistance of treated and untreated wool to bacterial attack. Ordinary after-chroming and treatment with cutch and chrome, or cutch and copper sulphate, impart a very considerable if not complete protection against bacteria and most moulds.

With certain fungi imperfecti—*e.g.*, *Fusarium* spp. and certain actinomycetes—the effect is less marked, though still considerable. An effective chemical treatment which does not result in discoloration of the wool has not yet been produced. The trypsin technique also shows that certain hairs—*e.g.*, human hair, pig hair, and calf hair—are very resistant to microbiological disintegration, whereas certain sheep's wools and camel hair are readily broken down.

For a further account of the microbiology of wool reference may be made to Burgess (1934) and Bartsch (1931, 1932).

Many cases of woolsorter's disease have occurred amongst workers handling imported goat and other hairs. It is now compulsory for those types of materials which are more liable to contamination to be sterilised by formalin treatment at the Government station at Liverpool. The causal organism, the spore-forming *Bacillus anthracis*, is satisfactorily dealt with by this means.

Pathogenic fungi associated with ringworm and similar diseases may be found actually within the keratinised hair. It may be that penetration occurs at the base of the follicle before keratinisation takes place, although species of *Achorion* causing favus are said to enter the hair without growing down into the follicle.

Other Textile Fibres.

Most of the vegetable fibres react to micro-organisms in a somewhat similar manner to cotton, but the nitrogenous impurities—*e.g.*, of **flax**—are usually removed in the processes preceding spinning and weaving. Consequently mildew problems are less serious in the linen industry than the cotton industry.

Jute is a heavily lignified fibre, but is by no means immune from fungal and bacterial attack. For safe storage the regain should not exceed 17 per cent. (Galloway, 1939). This corresponds to a relative humidity of 80 per cent.

"Heart damage" in jute is attack within the bale, probably mainly bacterial, and occurs only when the jute is abnormally damp—*cf.* Finlow (1918), Schepman (1927).

Hemp is used for better quality fishing nets, but when these are left damp for prolonged periods bacterial rotting occurs, especially at the knots. Tarring, cutching, or more modern

treatments with copper soaps, all tend to prolong the life of the nets—*cf.* Atkins (1930), Robertson (1931).

The fungi causing deterioration of **Manila hemp** are described by Serranio (quoted by Wardlaw, 1935), who finds *Asp. flavus*, *Asp. niger* and *Asp. fumigatus* the most important. Fibre should not contain more than 11 to 12 per cent. moisture.

Rayons may be of cellulose or of cellulose acetate, and the latter type is remarkable for its resistance to microbiological action. Thaysen (1932) advocates acetylation as a means of rendering cellulosic materials rot proof.

Natural Silk.—Here the fibre is composed of the protein fibroin coated with silk gum, or sericin. Micro-organisms develop more readily on the raw silk than after it is degummed, and are sometimes a source of discoloration and loss of strength (Yendo, 1928). It may be noted that a whitish appearance on dyed silk fabrics, suggesting mildew, is sometimes traceable to a purely optical effect brought about by local damage to the silk filaments. Silk, like wool, appears to be very resistant to anaerobic decomposition.

A very complete summary of the literature on the deterioration of fabrics caused by micro-organisms is given by Thaysen and Bunker (1930).

Use of Enzymes in the Textile Industries.

Enzyme preparations are commonly employed for "desizing," or the removal of starch size from cloth in preparation for later finishing processes, such as bleaching or dyeing. It is desirable that the preparation used should have some proteolytic power in addition to its ability to liquefy starch. Saccharifying power is of no importance, since all that is required is that the starch shall be rendered soluble.

Malt enzymes are probably the usual form employed, but many firms prefer to use commercial starch-removing enzymes obtained from *Aspergillus oryzæ* or from bacterial sources.

For the purpose of degumming silk—a process usually carried out by chemical means—the use of bacterial cultures has been recommended by various workers.

Certain patents have been taken out for the use of enzyme preparations of pectinase for retting, and this seems a process that might be capable of development. Organisms such as

Erwinia carotovora (*Bact. carotovorum*) or certain species of *Rhizopus* might be used as a source of pectinase.

REFERENCES

ATKINS, W. R. G. (1930): J. Marine Biol. Lab., **16**, 583.
BARTSCH, J. (1931): Textilber., **12**, 760.
—— (1932): *Ibid.*, **13**, 21.
BRIGHT, T. B. (1926): J. Text. Inst. **17**, 396T.
BRIGHT, T. B., MORRIS, L. E., AND SUMMERS, F. (1934): J. Text. Inst., **15**, 547T.
BURGESS, R. (1934): J. Soc. Dyers and Col., **50**, 138.
—— (1934*a*): J. Text. Inst., **25**, 289T, 391T.
—— AND RIMINGTON, C. (1929): J. Roy. Micros. Soc., **49**, 341.
DESMEDT, R. (1933): Het Vlas, **13**, 161.
EYRE, J. V., AND NODDER, C. R. (1924): J. Text. Inst., **14**, 237T.
FARGHER, R. G., GALLOWAY, L. D., AND PROBERT, M. E. (1930): J. Text. Inst., **21**, 245T.
FINLOW, R. S. (1918): Mem. Dept. Agric. India (Chem. Ser.), **5**, No. 2.
FLEMING, N., AND THAYSEN, A. C. (1921): Biochem. J., **15**, 407.
GALLOWAY, L. D. (1930): J. Text. Inst., **21**, 277T.
—— (1931): *Ibid.*, **22**, 494T.
—— (1939): *Ibid.*, **30**, 127T.
NEILL, J. C., AND TRAVERS, E. (1938): N. Zealand J. Sci. Tech., **19**, 646.
NOPITSCH, M. (1933): Textilber., **14**, 139.
ROBERTSON, A. C. (1931): Ind. Eng. Chem., **23**, 1093.
ROSSI, G. (1916): Bull. Agric. Intell., **7**, 1067.
SCHEPMANN, W. (1927): Textilber., **8**, 237.
SEARLE, G. O. (1929): J. Text. Inst., **20**, 162T.
THAYSEN, A. C. (1932): Eng. Pat. 399,952.
—— AND BUNKER, H. J. (1927): The Microbiology of Celluloses, etc. London.
—— —— (1930): Second Report Fabrics Co-ord. Res. Committee. H.M. Stationery Office.
WARDLAW, C. W. (1935): Diseases of the Banana. London.
YENDO, Y. (1928): Abst. Exp. Sta. Record, **58**, 392.

CHAPTER XII

HYGIENE

PUBLIC health microbiology, although a vast subject, can be allotted only a single chapter in the present summary, and attention will be devoted mainly to applications of industrial hygiene. Fuller information on the pathogenic bacteria will be found in textbooks such as those of Muir and Ritchie (1932), Topley and Wilson (1936), or in the series of volumes published for the Medical Research Council (1931). For veterinary bacteriology see Gaiger and Davies (1938).

Micro-organisms play an extremely important part in the maintenance of the public health. They are responsible for a large number of deaths each year and for a vast amount of illness. The organisms may produce diseases which are highly infectious, and therefore tend to occur in epidemic form —*e.g.*, influenza—or in conditions which affect the individual only—*e.g.*, furunculosis.

The havoc wrought in the past has been responsible for the setting up of organisations for the prevention and control of outbreaks.

In 1907 there was established the Office International d'Hygiène publique in Paris to deal with the international control of infectious diseases. In 1926 the International Sanitary Convention attempted to establish a standard procedure in the different countries. In England the Port Sanitary Regulations, 1933, followed as a result.

Our own Ministry of Health had its origin in an attempt in the nineteenth century to control outbreaks of infectious diseases. Since then the scope of the Ministry has been extended, but its constitution and much of its work adequately reflect the part played by micro-organisms in public health. Of the six sections of the Ministry in England, Section I. is concerned with General Health and Epidemiology, section III. with Tuberculosis and Venereal Diseases, Section IV. with the Supervision of Food Supplies, and Section VI. with Sanitary Administration in Relation to Infectious Diseases.

A considerable amount of legislation has been passed with the object of preventing and controlling infectious diseases. The work is largely carried out by local authorities under the guidance of their Medical Officers of Health, and is under the general supervision of the Ministry of Health.

Infection and Immunity.

The term infection, when used in its medical sense, implies the penetration of the tissues by micro-organisms and their multiplication within the tissues. It is an association between host and organism in which there is damage to the tissues of the host.

A healthy animal body normally contains a large and varied bacterial flora, particularly in the digestive tract, where *Bact. coli* flourishes in the large intestine. The mouth, also, contains a mixed collection of cocci, rods and spirochætes which are normally harmless. Fundamentally, however, these regions are external to the real body, whose surfaces are protected by a covering of skin or of mucous membrane, both of which are resistant to infection.

Many pathogenic bacteria produce poisonous substances called toxins. These may be *endotoxins*, retained within the micro-organism until the latter dies, or *exotoxins*, which diffuse into the body tissues and consequently produce symptoms soon after infection has occurred.

In some instances the entrance of pathogenic organisms to the tissues of an animal is not attended by disease, and the animal is said to be immune. The many types of this individual immunity are described by Topley and Wilson (1936). Immunity to a particular disease may be acquired actively or passively.

Active Immunity.—In certain diseases—*e.g.*, diphtheria—the immunity may be of an active kind. That is, the body tissues have produced substances (antibodies) which render them immune from attack by a specific organism. This is often the result of an actual attack of the disease. Indeed, a body's most important defensive system may be the accumulation of substances of this nature following intermittent subclinical doses of infection.

Active immunity may also be conferred by a process of active immunisation which consists of the introduction into the body

of a dose of toxin—*e.g.*, diphtheria; or of dead organisms —*e.g.*, typhoid; or of living attenuated (weakened) virus—*e.g.*, the vaccine for smallpox, such that, while there is no serious illness produced, immunity results. Substances such as the above, which stimulate the production of antibodies, are called *antigens*.

Passive Immunity.—In this type the substances capable of conferring immunity are introduced from without—*e.g.*, diphtheria antitoxin, scarlet fever antitoxin. This immunity is short-lived. Such antitoxins are contained in blood serum usually obtained from some animal which has been subjected to increasing doses of antigen.

Other natural lines of defence, in addition to antibody production, are—(i.) The phagocytic (bacteria-ingesting) action of certain leucocytes of the blood. The " pus " resulting from infection by *Staphylococcus aureus* consists largely of leucocytes, dead bacteria, and exuded lymph. (ii.) The localisation of the infecting agent, such as occurs in tuberculosis. A cross-section of an adult tubercle shows a central zone of degenerated tissue surrounded by zones of leucocytes and other cells, and finally an outer layer of fibrous tissue.

Besides the administration of antibodies, the fight against an infection may sometimes be resisted by improving the general health and hence the resistance of the individual by change of environment, diet or mental outlook, or by chemical or other treatment.

Source of Infection.—In the case of the most frequent diseases of man the source of infection is man himself. The disease is often conveyed from one person to another by means of " droplet infection "; that is, during coughing and sneezing a spray of saliva containing pathogenic bacteria or virus is ejected and this enters the naso-pharynx of the new subject—*e.g.*, influenza, measles.

Contaminated food and water are fairly common sources of infection, the resulting disease being usually of intestinal nature, though sometimes it becomes generalised.

Some diseases, like syphilis and ringworm, are transmitted by contact with diseased individuals.

Soil is occasionally responsible for cases of tetanus, while rats, mice, fleas, flies, mosquitoes, etc., may all play a part in the spread of disease.

A serious obstacle to the control of certain epidemic diseases is the " carrier," the individual who, while showing none of the symptoms of the disease, unconsciously disseminates the infective agent.

Micro-organisms Pathogenic to Man and Animals.

As pointed out in an earlier chapter, the development of bacteriology was for many years principally based on the study of pathogenic bacteria. The " germ theory " of disease led to marvellous advances in medicine; on the other hand, it has implanted in the public mind a somewhat exaggerated terror of " germs " in general, which is still exploited by the advertisers of certain soaps and disinfectants.

Bacterial Diseases of man and animals are more common than diseases caused by fungi, thus reversing the position found in the plant world. For a description of the organisms the reader is referred to the textbooks mentioned at the beginning of this chapter.

Among the more serious diseases due to bacteria may be mentioned bubonic plague, cholera, tetanus and anthrax.

Diseases caused by *Actinomycetes* are referred to in Chapter III.

Viruses ; Protozoa.—Among the diseases due to viruses—or at any rate to ultra-microscopic organisms—are measles, mumps and hydrophobia, whilst protozoa are responsible for malaria, sleeping sickness and certain types of dysentery.

Fungal Diseases.—These are often referred to as *mycoses*—*e.g.*, dermatomycoses, etc. In addition to the typical mycoses, bronchial troubles are sometimes brought about by inhalation of mould spores; it is not clear whether this is a purely physical effect or due to histamine compounds. *Aspergillus fumigatus* develops in the air sacs of domestic birds.

Sporotrichosis in man often follows wounds, and produces a row of hard, raised lumps. Internal administration of iodides usually effects a cure.

Blastomycosis also follows wounds, and produces raised sores that discharge pus.

The typical *Dermatomycoses* include ringworm, " dhobi itch," and favus.

Thrush was at one time a common disease of infants, and is caused by *Monilia albicans*.

Food and Disease.

Illness caused by the consumption of a certain food may be due to the natural toxicity of the material—*e.g.*, the mushroom *Amanita phalloides*—to contamination by poisonous chemicals, or to bacterial contamination. Only the last type will be considered here.

Milk-Borne Diseases.—Of the foods acting as disseminators of pathogenic bacteria, milk is by far the most important. The danger of tuberculosis infection from cow's milk is gradually being dealt with by the eradication of infected animals and the pasteurisation of bulked milk. Undulant fever (*Brucellosis*), common in many parts of the world, is often traceable to goat's milk or to cow's milk, and in such cases can only be prevented by strict control of the milk supply. Other diseases —*e.g.*, typhoid, paratyphoid, dysentery, diphtheria, scarlet fever and septic sore throat—may be spread through milk supplies, the infection usually being due to handling by an infected human being (see Jameson and Parkinson, 1934, and pamphlet issued by the Economic Advisory Council, 1934). The dangers of an infected milk supply are now so fully recognised that local authorities have full powers to enforce strict cleanliness and the use of modern hygienic methods by all milk producers.

Cases of food poisoning have been found to be due to the toxic products of bacteria, notably species of *Salmonella*, carried in milk products such as cheese and ice cream.

Meat and Meat Products.—Every carcass is inspected by the local authority as soon as possible after slaughter, and, according to its condition or the nature of any disease process, the meat is liable to be condemned.

Meat products have frequently been found to be vehicles of infection leading to food poisoning. It was formerly held that many cases of food poisoning were due to the ingestion of *ptomaines*, complex chemical substances resulting from the breakdown of proteins by saprophytic anaerobic bacteria. The work of Savage (1920) and others has shown that the toxic substances causing food poisoning are of a different nature, and most probably result from infection by the *Salmonella* group of bacteria. These organisms multiply readily in foodstuffs, particularly canned meats, and their heat-resisting

(100° C.) endotoxins are taken in with the food. In other cases, such as made-up meats, food poisoning is due more to the ingestion of living *Salmonella* organisms than their accompanying toxins. Nevertheless, in many outbreaks of food poisoning, *Salmonella* infection has not been demonstrated, and it is probable that such illness was caused by consumption of poisonous substances formed in food by the growth of other types of bacteria. Incidentally, species of *Salmonella*—*e.g.*, *S. enteritidis* var. *danysz*—are used by man as the active constituent of certain rat " poisons."

For information regarding the bacteriological examination of suspected food samples, see Ministry of Health memorandum (1935) and Hunwicke (1931).

Fish and Shell Fish.—Decomposing fish may cause gastric disturbances, while shell fish—*e.g.*, oysters, mussels, cockles, etc.—may convey to man such diseases as typhoid, paratyphoid, and acute food poisoning, the source of the pathogenic bacteria being the shell fish layings—*e.g.*, mud estuaries contaminated by human fæces. Bacteriological examination is confined mainly to the *Bact. coli* content. Research, so far as it has gone, indicates that the safety of shell fish depends on the freedom of their habitat from fæcal contamination.

Other Sources of Food Poisoning.—Fresh fruit and vegetables, and canned or otherwise processed vegetable products, have also been associated with food poisoning. Watercress, like shell fish, is liable to contamination by sewage, and may carry disease organisms. Eggs, especially duck eggs, infected with *Salmonella aertrycke* and *S. enteritidis* have caused food poisoning in man.

Mice and other vermin infected with *Salmonella* organisms are a source of danger in connection with the food of man, farm animals and poultry through their contaminated excrement.

Botulism.—The outstanding example of food poisoning due to a bacterial toxin is botulism, which has so far fortunately been of rare occurrence in Britain. The causal organism, *Clostridium botulinum*, is a large, feebly motile, anaerobic, Gram-positive rod, forming spores which are highly resistant to heat, and hence occasionally escape sterilisation in non-acid canned foods, such as meat and vegetables. Although not itself pathogenic, the organism when growing saprophytically on the food produces an extremely powerful toxin which is

almost invariably fatal if any appreciable amount is consumed. The toxin is, however, readily destroyed by five minutes at 100° C., or thirty minutes at 80° C., and food about which there is the slightest suspicion of "taint" should therefore be cooked immediately before it is eaten.

Water-Borne Diseases.—Of these the most important—*e.g.*, typhoid, dysentery and cholera—are due to the contamination of the water supply by sewage. The bacteriological examination of water will be dealt with later.

Air.

The atmosphere contains varying numbers of bacteria and of fungal and bacterial spores, either alone or attached to dust particles. These may be carried for considerable distances by air currents, but will eventually settle. Such organisms are mainly saprophytic soil organisms, including various species of *Aspergillus*, *Penicillium*, *Rhizopus* and other common moulds; yeasts and torulæ; sporing bacilli of the *subtilis-mycoides* type; micrococci; and various non-sporing yellow rods. At high elevations and at sea the amount of infection is, of course, much reduced.

Organisms pathogenic to plants may be carried by air for considerable distances. In the plains of India the rusts of cereals cannot survive the summer heat, and it now seems definitely established that annual infection by the cereal rusts depends entirely on spores carried by wind currents from the hills.

Organisms pathogenic to man are comparatively absent from air infections, being for the most part sensitive to low temperatures, to desiccation, and to sunlight. Thus human and animal diseases are rarely transmitted through air, the exceptions being certain droplet infections projected for several feet in the course of talking, coughing and sneezing. Crowded and badly ventilated vehicles and workrooms may therefore assist the spread of certain diseases.

From an industrial point of view the undesirability of air contamination in the food, dairy, and fermentation industries has been touched upon in the preceding chapters. In some of the older processes for making wine, vinegar, and certain cheese, natural infection was relied upon to produce the re-

quired organism. Modern methods, however, aim at securing more consistent results by the use of pure cultures or " starters," and excluding chance infection.

Estimation of Air Infections.—The enumeration of micro-organisms in air may be effected by aspirating the required volume of air either through a known quantity of sterile water or broth, or through plugs of a filtering substance, such as sterile cotton-wool, anhydrous sodium sulphate, sand, or sugar, which will collect the organisms. This is followed by plating out the dilutions of the liquid, or in the latter case the suspension made by shaking the filter plug with a litre of sterile tap water.

A more rapid estimation may be made by using Owen's (1926) jet extraction apparatus, with which the minute particles suspended in a known volume of the air under investigation are collected and stained on a small specified area of a clean cover-glass. The micro-organisms present may then be enumerated in a similar manner to that described for the Breed test (see p. 97).

Water.

All natural waters contain micro-organisms. The number depends on various factors, among which may be mentioned the amount of organic matter in solution, the temperature, the depth, and the rate of flow. Storage of water causes a decrease in the bacterial numbers, as the food supply becomes exhausted. Certain rivers—notably the Ganges—have an appreciable bactericidal action which may be due to the presence of a bacteriophage.

Bacteria occurring in water may be roughly classified into three groups: (i.) natural water bacteria, (ii.) soil bacteria, and (iii.) intestinal bacteria.

Natural Water Bacteria.—Reference has been made in previous chapters to the sulphur and iron bacteria, the latter being a frequent source of clogging in water pipes. Amongst other naturally occurring water types may be mentioned the genera *Achromobacter*, *Chromobacterium*, *Flavobacterium*, *Micrococcus*, *Pseudomonas*, *Sarcina*, *Spirillum*, *Serratia* and *Vibrio*. Most of these are comparatively innocuous, although *Ps. fluorescens* and certain *Micrococcus* spp. are of some importance in the dairy industry.

Sea water may contain large numbers of bacteria, especially near the shore. The bacterial microflora of the sea has been studied by Waksman and Carey (1935). It has been suggested by Zobell and Allen (1935) that marine bacteria play an important part in the fouling of submerged surfaces, a bacterial film preceding the attachment of other living bodies such as barnacles.

Soil Bacteria.—The principal types found are spore-forming rods such as *Bac. subtilis*. These sporing bacteria have strong proteolytic properties and an optimum temperature near blood heat. They are usually abundant after heavy rain.

Intestinal Bacteria are naturally of the greatest interest as regards public health. They comprise facultative anaerobes —*e.g.*, *Proteus* spp. and the *coli-aerogenes* group. Certain human and animal pathogenic types may also be present, as well as spore-forming anaerobes such as *Clostridium welchii* and *Cl. sporogenes*, and the fæcal streptococci. These organisms originate in the alimentary tract of animals and man, and find their way into water either directly or through soil drainage water and polluted drains. Their significance will be discussed in connection with the bacteriological examination of water. As a rule pathogenic organisms are short-lived, the environment being unfavourable, but in times of epidemics they may cause a continual contamination—*e.g.*, the organisms of typhoid (*Eberthella typhosa*), cholera (*Vibrio comma*) and dysentery (*Shigella* spp.).

The Removal of Micro-organisms from Drinking Waters. —This may be accomplished in several ways, utilising the principles of sedimentation and filtration, and coagulation by chemical means.

(i.) Sedimentation and filtration. In still water micro-organisms tend to settle out. Sedimentation is aided by bulking of water in reservoirs. During subsequent filtration the water is passed through successive layers of sand, gravel, pebbles and stones, to conducting channels below.

(ii.) Coagulation and filtration. Aluminium sulphate is added to the water. In the presence of lime salts flocculent masses of aluminium hydroxide are formed, and as these sink to the bottom they entangle all suspended particles. Sometimes bactericides—*e.g.*, hypochlorites—are added to the water after the coagulation process.

(iii.) Chemical treatment. Chlorine is added in the form of hypochlorites or liquid chlorine, about 0·2 part per million sufficing for normal filtered water. Where a considerable amount of organic matter is present, the addition of chlorine must be correspondingly increased. Chloramine at the rate of 0·2 to 0·3 p.p.m. and 0·3 to 0·5 p.p.m. of chlorine in filtered and unfiltered water respectively has been recommended. Combined treatment with chlorine and ammonium sulphate or cuprammonium, which produces chloramines, is also effective, especially if algæ are troublesome. The chlorine is added some twelve to eighteen hours after the addition of the ammonium compound. Chloramines appear to be preferable to chlorine where there is the possibility of the latter giving rise to an undesirable taste or to the formation of chlorphenols.

The use of 0·3 p.p.m. of each of the chemicals copper sulphate, ammonia and chlorine is suggested. Copper sulphate itself, applied at the rate of 0·5 p.p.m., destroys most algæ and protozoa, but as little as 0·14 p.p.m. is inimical to trout.

In addition to its use for drinking-water supplies, chlorine is particularly applicable to the purification of swimming-baths, which rapidly become infected with nose- and mouth-infecting streptococci and with fæcal organisms. The chlorine content should be maintained at about 0·4 p.p.m. (Mallmann, 1935).

Iron bacteria are destroyed by six to twelve hours' treatment with alkaline sodium hypochlorite to give 1 p.p.m. of chlorine, followed by flushing.

The presence of chemicals, and especially copper, is obviously undesirable in water used for boilers and in the general supply of food, textile and other factories. Next to steam or boiling water, a hypochlorite solution, if carefully used, is probably the best for sterilising plant in the food industries.

(iv.) Other methods of purification and sterilisation, applicable to special cases, include filtration through porous filters of the bacterial filter candle type, exposure to ultra-violet rays, " catadyn " treatment (silver on sand), and treatment with ozone. For ordinary purposes water is most simply and effectively sterilised by boiling.

For further details regarding the purification of water supplies reference may be made to Thresh, Beale and Suckling

(1933), and the summaries on water pollution research published monthly by the Department of Scientific and Industrial Research.

Bacteriological Examination of Water.—Details of standard technique will be found in Report No. 71 of the Ministry of Health (1934). The isolation of specific pathogenic bacteria is a matter for specially equipped laboratories and staff, and the normal routine bacteriological examination of water is confined usually to standard tests such as the following:

(i.) Nutrient agar count after three days at 20°-22° C. This gives an indication of the amount of organic food present capable of supporting bacterial growth, and of foreign material such as soil and dust that has entered the water.

(ii.) Nutrient agar count after two days at 37° C. The figures obtained are usually considerably less than for (i.), but they include soil, sewage and intestinal organisms, and may hence be used as an index of purity. If the ratio (i.) : (ii.) is less than 10, the water may be polluted, but no real value can be attached to a single observation, and the sampling should be repeated at regular intervals. The ratio has no significance in chlorinated water.

(iii.) *Coli-aerogenes* test in MacConkey broth (the "presumptive coli" test). Quantities of 50, 25, 10, 5, 1, and 0·1 ml. of water are added to tubes of MacConkey broth; in the first four cases an equivalent of double strength broth is used. The flasks or tubes are provided with Durham fermentation tubes. After one or two days' incubation a positive acid and gas reaction indicates the presence of organisms of the *coli-aerogenes* group. If *Bact. coli* is found in 1 ml., the water is open to suspicion and should be considered as unfit for domestic use. The test is carried out in quadruplicate.

The *coli-aerogenes* bacteria resemble one another closely, but may be differentiated by the use of a group of tests, including sugar fermentation, indole production, liquefaction of gelatin, growth in citrate medium, and the Voges-Proskauer and methyl red tests (*cf.* Chapter VI.). Whilst *Bact. coli* is typically of intestinal origin, *Bact. aerogenes* is a saprophyte on vegetable tissues and in soil. In America and in the Tropics this difference of origin is very marked, and special plating media are used to distinguish between the two types.

In Britain, however, the frequent presence of *Bact. aerogenes* in sewage and fæcal matter, and its tendency to survive longer in water than *Bact. coli*, are taken as sufficient justification for its inclusion with the true coliform organisms in indicating fæcal contamination. The confirmatory or differentiating tests are initiated by plating a small quantity of the highest dilution of the broth showing acid and gas on to MacConkey agar. Red colonies, denoting acid production, are sub-cultured into peptone water, which after short incubation provides the inoculum for the differential tests. The following table indicates the reaction of the common acid and gas *coli-aerogenes* organisms:

	Indole.	*Voges-Proskauer.*	*Methyl Red.*	*Growth in Citrate.*
Bact. coli: ..				
Type I.	+	-	+	-
Type II.	-	-	+	-
Intermediate:				
Type I.	-	-	+	+
Type II.	+	-	+	+
Bact. aerogenes:				
Type I.	-	+	-	+
Type II.	+	+	-	+

Truly fæcal strains—e.g., *Bact. coli* Type I.—are best picked out by the production of acid and gas in MacConkey broth at 44° C. (Wilson *et al.*, 1935).

These tests may be supplemented where necessary by tests for the fæcal streptococci and the anaerobic spore-forming *Clostridium welchii* and *Cl. sporogenes*. In the first case 1 ml. of the culture in MacConkey broth is carefully diluted in 9 ml. of sterile water at 60° C., and the tube heated in the water bath at 60° C. for fifteen minutes. One drop of the liquid is then spread over the surface of a dried MacConkey agar plate by means of a sterile bent glass rod. After twenty-four hours at 37° C., any streptococci which, unlike the *coli-aerogenes* bacteria, resist the heating, appear as small, intensely red colonies. Some of these are tested microscopically and by biochemical tests. A positive reaction for fæcal streptococci gives confirmation to a doubtful *coli-aerogenes* test.

Clostridium welchii may be present in water in the resistant

spore form. It is detected by adding 100, 20 and 10 ml. quantities of water to sterile litmus milk, the mixture being heated to 80° C. for fifteen minutes and incubated anaerobically. If whole milk is used the layer of fat will suffice to render conditions below it anaerobic, otherwise a layer of sterile mineral oil or vaseline will serve this purpose. A positive reaction is indicated by the development of the characteristic stormy fermentation within sixteen to seventy-two hours—the milk is curdled and the curd broken up by very rapid evolution of gas. A further method is to inoculate 40 ml. of the water into 40 ml. of melted Wilson-Blair medium (see Chapter VI.). This is poured into a large petri dish and incubated aerobically for twenty-four to thirty-six hours at 37° C., or preferably at 45° C. Large black colonies more than 3 mm. in diameter are those of *Cl. welchii*. Wilson (see Min. of Health, 1934) regards *Cl. welchii* as essentially fæcal in origin, and the derivation of only one large black colony from 40 ml. of water may be taken as indicating fæcal pollution when no positive result was obtained for *coli-aerogenes* in MacConkey broth.

Sewage.

Sewage is derived from a multiplicity of sources, and is hence of very complex nature, containing both decomposable organic matter providing nutriment for micro-organisms and chemicals that may tend to inhibit microbiological decomposition. In towns the sewage may be markedly affected by the nature of the local industries. Thus dairies tend to give an acid effluent and soda works an alkaline one. The flora is itself heterogeneous, and includes many types of bacteria, fungi and protozoa.

In the disposal of sewage two aspects are of prime importance: (i.) conversion of the complex mixture of substances into a simple innocuous form that can be safely discharged into the drainage system; (ii.) removal and destruction of pathogenic organisms.

The bacterial decompositions form a varied series of overlapping reactions, and are predominantly aerobic or anaerobic according to the conditions obtaining in the sewage. Anaerobic bacteria are particularly active in the decomposition of urea, proteins, cellulose and fats. Pathogenic organisms may be

present in the sewage, especially during epidemics of disease; most die off fairly soon, but proof of their complete destruction is difficult to obtain owing to the difficulty of detecting such organisms when highly diluted.

The **disposal of sewage** is secured by creating conditions favouring microbiological growth. Broadly, there are two methods, the use of filters and of septic tanks. Filters consist of crushed material—*e.g.*, stone, coke, gravel or sand—over which the sewage is allowed to trickle. Chemical absorption takes place, and a gelatinous bacterial film develops that holds back suspended matter and also forms a stratum for the aerobic decomposition of organic matter. The filter requires constant attention, being adversely affected by flooding, cold, or the presence of harmful chemicals. Heat evolved during fermentation helps to prevent freezing. A simple form of filtering device is often employed in village " sewage farms " where sewage is deflected into the soil or to a tile drain in the soil. In all cases free access of air is essential, and filter beds require periodical cleaning.

In the second method, the sewage, interrupted by baffle boards that also trap the grease, flows slowly through a large or small " septic tank." The tank contains a predominating microbial flora that effects an anaerobic decomposition of the organic matter. A surface scum of aerobic bacteria, fungi, and yeasts assists to maintain anaerobic conditions below. The liquid on leaving the tank is discharged into the drainage system directly or after passage through some form of filter.

A modification of the septic tank system is that in which compressed air is blown through the liquid, thus " activating " the sludge. In this case the decomposition process is entirely aerobic. The extra cost is repaid by greater compactness, a reduction in offensive odour, increased efficiency, and the marked manurial value of the sludge (see Fowler, 1934).

In the recently installed West Middlesex drainage purification works the sewage, after removal of the grit and heavy solid matters by screening and sedimentation, is subjected to the activated sludge process. After further purification treatment the clear liquid is discharged while the sludge is made to undergo a bacterial digestion by which one half of the solid matter present in the crude material is converted into methane, which is used to generate power for the operation of the works.

Before discharge into streams, the liquor may be chemically treated—*e.g.*, with calcium hypochlorite to the extent of 1 to 5 parts per million. This treatment destroys almost all the remaining bacteria, whereas chemical treatment of the original sewage is prohibitive on account of the high protein content.

Bacteriological Examination of Sewage.—This is conducted on similar lines to the examination of potable waters, using higher dilutions of the samples. References to recent work on sewage disposal will be found in the abstracts on water pollution research issued monthly by the D.S.I.R.

Industrial Wastes.

The effluent from certain industries presents a serious problem, and in many cases the industry is required to ensure its safe disposal. Chemical wastes may be poisonous to fish, domestic animals, or even to local residents, and may interfere with the normal microbiological decomposition of sewage. Wastes from brewing and dairy industries are rich in carbohydrates leading to acid fermentations and undesirable fungal growth during the aerobic disposal of sewage. The commonest filamentous organism in organic effluents is *Sphærotilus natans* (see Butcher, 1932). Such organisms often lead to pipe stoppages, trouble in condensers, etc. Phenols may be oxidised by bacteria; iron salts may stimulate iron bacteria, and so forth. Mineral oils are removed with difficulty, and inhibit both bacterial life in the filters and fish life in the streams. Certain organic wastes like fat from wool-scouring processes offer considerable resistance to bacterial decomposition, such resistance being reduced by emulsification and dilution of the fatty material.

An interesting example of waste treatment is the Bradford treatment of sewage, which consists of half domestic and half industrial waste (wool scouring and dye liquors, etc.). After removing coarse ingredients in settling tanks provided with baffle boards, the sewage, when necessary, is acidified to pH 6 to precipitate grease. The liquid then passes to bacterial filters made of coal, while the grease is recovered and the sludge pressed and ground into a powder of manurial value.

Further examples are the treatment of the effluents from sugar beet and dairy factories which have been investigated at

Rothamsted. The sugar beet effluent is passed over a finely graded clinker filter so that the sugar is completely oxidised. Special attention is paid to the suspended mud. A 90 per cent. purification is obtained (D.S.I.R., Water Pollution Board, 1928-29, Papers Nos. 36 and 41).

In the case of dairy wastes, successful results are being obtained by subjecting the liquid after settling either to the activated sludge process or to biological oxidation on two percolating filters in series, the order of the filters being periodically reversed (Parker, 1936). The disposal of dairy wastes is also discussed by Barritt and Muers (1936).

REFERENCES

Barritt, N. W., and Muers, M. M. (1936): J. Soc. Chem. Ind., **55**, 48T and 71T.

Burdon, K. L. (1939): Medical Microbiology. New York.

Butcher, R. W. (1932): Trans. Brit. Myc. Soc., **17**, 112.

Dept. Sci. Ind. Research (1928-29): Water Pollution Research. Papers 36 and 41. H.M. Stationery Office.

Economic Advisory Council (1934): Committee on Cattle Diseases: Diseases carried by Milk. H.M. Stationery Office.

Fowler, G. J. (1934): Introduction to the Biochemistry of Nitrogen Conservation. London.

Gaiger, S. H., and Davies, G. O. (1938): Veterinary Pathology and Bacteriology. London.

Hunwicke, R. F. (1931): Essentials of Bacteriological Technique. London.

Jameson, W. W., and Parkinson, G. S. (1934): A Synopsis of Hygiene. London.

Mallmann, W. L. (1935): J. Bact., **29**, 26.

Medical Research Council (1931): A System of Bacteriology. 9 vols. London.

Ministry of Health (1934): Reps. on Public Health and Med. Subjects, No. 71. Bacteriological Examination of Water Supplies. H.M. Stationery Office.

—— (1935): Memo. 188/Med. Food Poisoning. H.M. Stationery Office.

Muir, R., and Ritchie, J. (1932): Manual of Bacteriology. London.

Owens, J. S. (1926): Analyst, **51**, 2.

Parker, A. (1936): Chemistry and Industry, **55**, 515.

Savage, W. G. (1920): Food Poisoning and Food Infection. London.

Thresh, J. C., Beale, J. F., and Suckling, E. V. (1933): The Examination of Water and Water Supplies. London.

Topley, W. W. C., and Wilson, G. S. (1936): Principles of Bacteriology and Immunity. London.

Waksman, S. A., and Carey, C. L. (1935): J. Bact., **29**, 531 and 545.

Wilson, G. S., *et al.* (1935): Medical Research Council. The Bacteriological Grading of Milk. H.M. Stationery Office.

Zobell, C. E., and Allen, E. C. (1935): J. Bact., **29**, 239.

CHAPTER XIII

AGRICULTURAL APPLICATIONS

ALTHOUGH this volume is intended primarily for the industrial microbiologist, it is impossible to ignore the agricultural aspects of mycology and bacteriology. Agriculture and industry cannot be separated into water-tight compartments.

In the long run all animal life is dependent on the plant kingdom—the most carnivorous of animals is a vegetarian by proxy—and the health of crop plants, trees, fruit and vegetables is therefore a matter of economic importance.

Crop Diseases.

The existence of crop diseases has long been known, and reference to what is translated as "blasting and mildew" may be found in Deuteronomy. With the development of the microscope it was observed that diseased plants showed various abnormal growths, but the realisation that such growths were often independent organisms dates back less than one hundred years. The Irish element in the eastern United States is largely a result of the emigration wave that followed the great potato famine of 1845-46 in Ireland. This famine was brought about by "late blight," and the attention thus focussed on this disease resulted in the discovery that blight was caused by a fungus, *Phytophthora infestans*. About the same time German botanists demonstrated that various other crop diseases were also brought about by fungi.

From these beginnings developed the science of plant pathology, and all agricultural departments of any magnitude are now provided with a mycologist or plant pathologist.

Various estimates have been made of the annual losses due to particular plant diseases, but such are largely guesswork. Plant parasites undoubtedly cause a steady reduction of yield, varied by an occasional severe epidemic. Wild plants also

have their fungal parasites to a less extent, but the cultivated plants, in addition to being less hardy, are grown under mass conditions that favour epidemic outbreaks.

In addition to the diseases caused by fungi, plant ailments may be brought about by bacteria, by viruses, or by non-parasitic causes connected with nutrition or climate. Insects and allied parasites are in modern usage termed pests, not diseases. A list of reference books on plant diseases is given at the end of this chapter.

It is perhaps advisable to emphasise here that the presence of bacteria or fungi in diseased plant tissues does not necessarily indicate that they are the cause of the diseased condition. Many of the organisms found on diseased plant tissues are purely saprophytic. Before a micro-organism can be regarded as the specific cause of a certain disease, the requirements of Koch's "postulates" as formulated for human pathology should normally be fulfilled.

(1) The organism must be constantly present in typical diseased tissues.

(2) The organism should be isolated in pure culture.

(3) The isolated organism when used to infect healthy plants must produce the disease in a high percentage of cases, the uninfected plants remaining healthy.

Difficulties arise in many instances. Postulate 2, strictly speaking, is possible only when the organism is capable of growing on artificial media, but an approximation to it can usually be made. Postulate 3 may be possible only when exactly the right condition of temperature, humidity, etc., is provided. To secure infection it may be necessary to wound the host plant, or to weaken its vitality in some way.

Fungi.—The fungi causing plant disease are so numerous and varied that only the most general observations can be made about them here. Some can be grown on artificial media in the laboratory, whilst others—*e.g.*, the rusts—grow only on living plant tissues. They may be strictly specific to a single host, or capable of infecting a number of different hosts. Identification of known plant parasites is facilitated by the existence of "host indexes"—*e.g.*, Saccardo (1882-1931), Oudemans (1919-1924), Seymour (1929), which list the fungi recorded for each host plant. Reference has been made in Chapter II. to the more important pathogenic fungi. Among

the *Phycomycetes*, the downy mildews (*Peronosporaceæ*) develop a non-septate mycelium within the tissues of the host plants, with aerial conidiophores that give a downy appearance to the surface tissues. In addition to the blight of potato already mentioned, other serious diseases, such as vine mildew, hop downy mildew, various "damping off" diseases, and the numerous diseases caused by species of *Phytophthora*, all belong to this group.

Among the *Ascomycetes*, the powdery mildews (*Erysiphaceæ*) differ from the downy mildews in that the mycelium is septate, and, apart from short, penetrating, food-absorbing hyphæ (haustoria), only occurs outside the host tissues from which it draws its nourishment. The *Pyrenomycetes* have thick-walled, flask-shaped, spore-containing bodies called perithecia, and include several serious parasites of fruit trees, such as *Nectria galligena*, the cause of apple canker.

The *Basidiomycetes* include two great groups of characteristic plant parasites, the smuts and the rusts. The smuts derive their name from the sooty spore masses that they produce; *Ustilago*, *Urocystis* and *Tilletia* are the most important genera, the last-named, including *Tilletia caries*, being the cause of bunt in wheat, the grains of which become filled with a black and fishy-smelling mass of spores. Most other cereals and grasses are also affected by smut diseases. The rusts (Grove, 1913; Arthur, 1929) are still more important diseases on cereals and other crops, and are so called because of the yellow, orange or brown spore pustules which they produce at one stage in their life history. The rusts of wheat are the best known and the most serious economically. Of other rust fungi, mention may be made of *Hemileia vastatrix*, which at one time destroyed the coffee industry in Ceylon, and indirectly led to the introduction of quinine from the new world to the old. Other important parasites belonging to the *Basidiomycetes* are the various bracket fungi causing damage to timber trees.

The vast group of *Fungi imperfecti*, often dismissed in a few pages in the textbooks on mycology, cannot be ignored in the treatise on plant diseases. Many anthracnoses, wilts, foot-rots, leaf spots and stem lesions are caused by members of this group.

Bacteria.—It was at one time considered that bacterial parasites were confined to animals; but just as the list of

animal diseases has been found to include many ailments due to fungi, so an increasing number of plant diseases have now been traced to bacteria. Pioneer work in this field was carried out from 1905 by Erwin F. Smith (1920), and later Elliott (1930) was able to list over 150 species of bacteria known to be parasitic on plants. Bacterial wilts are often caused by the vascular bundles becoming choked up by masses of bacteria; one of the best known examples is *Phytomonas (Bact.) solanacearum* that affects potato, tomato and tobacco. Gall formation may be a symptom of bacterial attack, as in crown gall, *Phytomonas (Bact.) tumefaciens*. Or the tissues may be attacked, as in soft rot, *Erwinia carotovora (Bact. carotovorum)* of turnips and allied crops, and in fire blight, *Erwinia amylovora (Bact. amylovorum)* of apples and pears.

Species of *Actinomycetes* may cause diseases of plants—*e.g.*, the common scab of potatoes.

Viruses.—Virus diseases of plants have been known to exist since the discovery at the end of the last century that tobacco mosaic could be transmitted by the pricking of infected juice into a healthy plant, even when the juice was first rendered free from micro-organisms by passing it through a Chamberland filter. In recent years increasing attention has been devoted to the study of virus diseases, especially those of tobacco and potato. It is now realised that different symptoms —mosaic, leaf curl, dwarfing, etc.—may be induced by the action of a single virus or of a combination of two or more viruses.

The criteria for the recognition of a plant virus are that it should be capable of multiplying indefinitely in suitable host tissues, and that it should be transmissible to a healthy plant either by direct inoculation or by grafting, though no visible organism can be detected. Not all plant viruses are filtrable.

The nature of viruses is still uncertain; claims have been made that certain viruses are the invisible "cyclostages" of bacteria whose visible stage can be obtained under suitable conditions of culture. The more commonly held view is that no organisms are involved; Stanley (1935-36) claims to have isolated a crystalline protein possessing the properties of tobacco mosaic virus from diseased tobacco and tomato plants. This implies that a non-living chemical substance is capable of multiplying itself when in contact with suitable

living tissue; if this is confirmed, it may be assumed that such a process is far more widespread in nature than the few instances in which disease symptoms have called attention to it.

The Dissemination of Plant Diseases.

Plant disease fungi may be disseminated either as mycelium or as spores. The former, except when in the form of a sclerotium, is less capable of surviving unfavourable conditions, but is sometimes transported from place to place in a viable condition with fragments of infected soil or diseased plants. Spores are the more usual means of dissemination, for which purpose they are specially fitted by their lightness, their abundance, and in some cases their resistance to heat, cold and desiccation. They may be conveyed by wind, water, insects, birds or mammals, and by the activity of man. Seed from diseased plants is in many cases capable of carrying over the infection to the next year's crop.

In general, the spread of disease across large natural barriers, such as oceans or mountain ranges, is principally through the transport of infected material by human agency. It may be noted in this connection that recent increases in the amount and speed of transport have led to new dangers of disease dissemination, for plants as well as for animals.

The constant endeavour to produce better and better varieties of crop plants leads to repeated introduction of plants or seed from other countries, and it is important that the diseases of these plants should not be introduced with them. A plant disease in a new environment which it finds congenial may spread with alarming rapidity. Many serious epidemics have been attributed, though definite proof in such cases is difficult, to the introduction of infected plants from abroad—*e.g.*:

Chestnut Blight, in U.S.A., 1906.
Hollyhock Rust, in Europe, 1873.
Antirrhinum Rust, recent in England.
Dutch Elm disease, recent in England and U.S.A.

To check the spread of plant disease fungi, most countries have now introduced legislation in one or more of the following directions: (1) to restrict the movement of infected material within the country; (2) to prohibit entirely the importation of certain plants from certain countries; and (3) to refuse im-

portation of plant material unless accompanied by a certificate from a competent authority in the country of origin that they appear to be free from disease when despatched.

In addition to such legislation, special quarantine stations may be established near the port of entry, where plants under suspicion can be grown until pronounced free from disease. Plant pathology thus presents analogies to the restriction of movement of cattle suffering from foot-and-mouth disease, and to the quarantining of dogs entering England.

In America, where great changes in the natural vegetation have been comparatively recent and foreign introductions have been considerable, there are more plant diseases to the acre than in most other countries, and it is in that country that quarantine laws and phytopathological services have developed in the greatest abundance.

The Control of Plant Diseases.

Treatment of disease in plants presents somewhat different problems from those met with in animal medicine. There is, for example, nothing quite comparable to the blood stream in plants; this is an advantage in that many diseases tend to remain localised in the plant, but it is a disadvantage in that it makes chemical treatment more difficult to apply. Again, no warning symptoms can be reported by the patient, so that in many cases plant diseases are only recognisable as such when they are far advanced. There is, however, the important analogy with human medicine that the best hope of avoiding disease lies in the constitutional resistance of the plant or patient—an inherited resistance is best (see p. 164), and failing that the resistance acquired by healthy conditions of life.

Control measures may be grouped under three main headings: (i.) indirect control by cultural measures, (ii.) direct control of the parasite, and (iii.) the use of resistant varieties.

(i.) By cultural control is meant some appropriate modification in agricultural practice that tends to check the disease. Increasing the vigour of the plant is one such modification, although in some cases the addition of nitrogenous manures may be undesirable because it makes the plant less resistant and also increases the vigour of the parasite. In general, phosphate and especially potash increase resistance to fungal

parasites. Improved cleanliness of cultivation, the use of good seed, and the removal and burning of diseased plants as soon as they are observed, all help to restrict infection by disease organisms. Or the parasite may be evaded by avoidance of certain areas, by crop rotation, by the use of early or late varieties, and so forth.

(ii.) Direct parasite control is usually effected by means of chemical poisons (Martin, 1936). Occasionally other methods are used, such as the application of heat, or biological control by the encouragement of a parasite on the parasite. The chemical antiseptics principally used are sulphur and compounds of copper and mercury. The value of slightly soluble copper compounds is said to have been an accidental discovery; a vine grower, according to one version of the story, applied verdigris to his choicest grapes to suggest the appearance of mildew and thus prevent theft, and found to his surprise that vines so treated were very little affected by mildew. From this arose the standard Bordeaux mixture (copper sulphate and lime), and Burgundy mixture (copper sulphate and sodium carbonate), now so widely used to spray potatoes against blight, vines against mildew, etc. It is said that in the French vine-growing districts the cost of copper sulphate used annually for spraying exceeds £2,500,000. Sulphur, finely powdered and applied as a dust, has certain advantages for use on fruit trees; it is less likely to scorch foliage, and is effective against certain mildews and insects. The toxic principle of sulphur dusts has been variously attributed to sulphur dioxide, hydrogen sulphide, gaseous sulphur, pentathionic acid, and to sulphur in polysulphide form. Lime sulphur sprays and dusts are also commonly employed.

When spraying with fungicides, it is essential to see that the spray is carefully made up. A wetting agent to assist spreading and emulsification is usually added, and may be combined with an agent to secure adhesion. It is necessary to cover the leaves, etc., of the plant completely with the fluid, and for this purpose, especially in the case of orchard trees, a high pressure driving spray from a double nozzle is recommended (Turnbull, 1934-35). Often, to be effective, spraying must be done within narrow limits of time specified by either a stage in the growth of the tree or the fungus concerned, or of both. Copper sprays have proved their value, but are not ideal, as they leave a

very narrow margin between the least copper concentration which will check the fungus, and that which is sufficient to damage the plant. With recent advances in the study of organic antiseptics, it is to be anticipated that more new fungicides will be produced. Two which have recently found some application in agriculture and horticulture are salicylanilide and malachite green.

In addition to sprays and dusts applied to crop plants, two other forms of fungicidal control are employed—namely, seed disinfection and soil disinfection. Treatment of seed by steeping in solutions of formalin, copper sulphate, or other substances found appropriate to special cases, has long been practised. Against loose smut of wheat, where the fungal infection is *within* the seed, the only effective treatment known is immersion of the seed in hot water—of course, under carefully controlled conditions or the germination of the wheat may be seriously impaired. Since wet treatments involve either immediate sowing or thorough drying of the seed, treatments with dry " seed dusts " (mixed with the seed in a rotating drum) offer considerable advantages, and have largely displaced wet treatments. Copper carbonate is commonly used for seed dusting, and sometimes sulphur, whilst even more efficacious (though more expensive) are the dusts composed of organic mercury compounds mixed with an inert carrier, such as talc. Even formalin has been applied in dust form by absorption on finely powdered charcoal.

The third form of parasite control by fungicides is soil treatment with suitable chemicals. Formalin and copper sulphate are sometimes effective, but are apt to injure delicate seedlings or to interfere with the germination of seed planted too soon after soil treatment has been given. Phenolic compounds are safer in this respect. Recently chloropicrin has been claimed to give good results (Godfrey, 1936). Bordeaux mixture itself is a soil fungicide, the copper being present in a form not sufficiently soluble to check plant growth. A really cheap and effective soil fungicide would find considerable use in agriculture, and especially in horticulture. Sterilisation or partial sterilisation of soil by heat as a means of eliminating parasitic fungi and other pests, and otherwise improving the soil, is commonly practised in greenhouse work, but is too expensive for field use. An outdoor substitute,

naturally limited in its effectiveness, is the burning of stubble or trash on the field.

(iii.) The use of crop varieties resistant to disease represents in theory the ideal method of avoiding losses due to fungi and bacteria. Since the discovery by Biffen that resistance to yellow rust was a Mendelian character of wheat, the efforts of geneticists have been largely directed towards producing resistant crop types. Brooks (1935) has pointed out some of the difficulties in controlling plant diseases by the use of resistant varieties. It is usually very difficult to retain the good qualities of a susceptible type in the crosses made with a resistant type. Moreover, resistance to one disease does not necessarily mean resistance to other diseases, and in the case of a crop that is liable to suffer from various fungi, breeding for resistance becomes a complicated matter. A resistant strain may lose its resistance after continued cultivation, and conversely a parasite may itself evolve a fresh strain with greater powers of overcoming the resistance. In many cases it has been found that even when a resistant strain has been found satisfactory in one district, similar tests in another country or another part of the same country give quite different results. Many fungal parasites—*e.g.*, wheat rusts—that are morphologically indistinguishable are really composed of a number of " physiological strains " capable of infecting various groups of host varieties. Nevertheless, the testing of old and new varieties for resistance is an important part of the plant pathologist's work, and where a variety resistant to a certain disease is available, its use affords the best method of checking the disease. Certain varieties of potato, for instance, are immune from " wart " disease.

The Storage and Transport of Agricultural Products.

Of almost equal importance with the losses caused by diseases of the living plant are the losses caused by spoilage of fruit, vegetables, grain, dairy products, etc., due to fungi and bacteria. The general principles for the prevention of such damage have been mentioned in Chapters VIII. and IX. As regards fruit, the essential point is to avoid bruising as far as possible, since it is the damaged tissues that are most susceptible to saprophytic micro-organisms. Much work has

been done, mainly from a chemical point of view, on the development of acidity in stored grain. One of the principal factors—though not the only one concerned—is the development of mould fungi, which convert some of the starch to organic acid, in addition to giving a musty odour to the material. Hence dry storage is essential. Above a certain moisture content fungi begin to develop, and at a considerably higher moisture content bacterial growth occurs. The safe limit for storage corresponds to about 15 per cent. moisture content for most types of grain.

In warm and damp climates, the problem of grain storage is a serious one, and after a certain time of storage it is often necessary to sell off the grain at any price.

The storage of seed potatoes presents a somewhat similar problem, though in this case there is no question of reducing the moisture content to a limit at which fungi and bacteria will not grow, and as in the case of fruit storage, the best precaution is to avoid external damage as far as possible.

Soil Microbiology.

The bacteria and fungi harmful to plants are greatly outnumbered by other soil organisms whose importance in maintaining the fertility of the soil was early recognised in the classic researches of Winogradsky and Beijerinck. From an agricultural point of view, the bacteria and fungi of the soil are important in that they break down dead animal and vegetable material; some types convert this material into inorganic compounds available for plant growth, others lock it up in a form which is not readily utilised or waste it as gaseous compounds. It is therefore desirable to exercise some control over the soil population in order to secure predominance of the useful organisms. Microbiological activities are largely influenced by the physical and chemical nature of the soil and its plant and animal population. Under normal conditions the micro-flora of the soil is in a state of unstable equilibrium, but the addition of nutrient material results in a striking change in the activity and multiplication of various groups of organisms. For example, the normally active non-sporing bacteria become secondary to the rapidly developing sporing bacteria.

Caution must be exercised in drawing practical conclusions from laboratory experiments. The carbon-nitrogen ratio, already referred to in Chapter VII., is of great importance, its influence being determined by many of the factors above mentioned. For every 30 to 35 parts of cellulose decomposed, micro-organisms require 1 part of nitrogen. As regards decomposition of cellulose and hemi-celluloses, the fungi are probably more active than the bacteria. Work on soil microbiology, however, was for many years focussed principally on the bacteria of the soil. The relation between soil bacteria and soil protozoa is still a matter on which experts are not agreed.

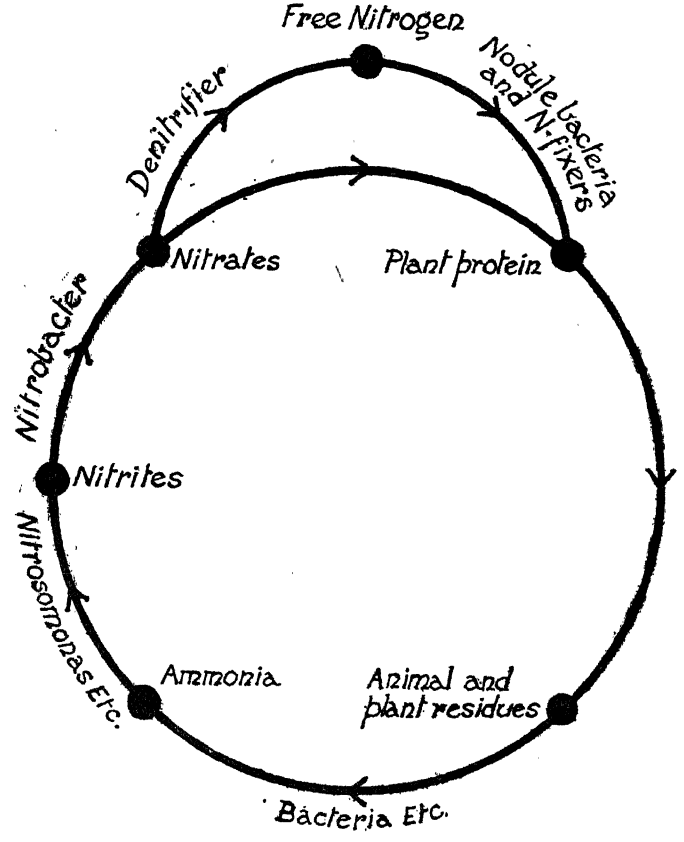
Soil Bacteria.—Fertile soil contains enormous numbers of bacteria of many different types. Only comparatively few of the predominating types have so far been studied fully, but it is clear that these are of great importance in maintaining soil fertility. Organic nitrogenous material is of no use as food to green plants, which require their nitrogen as nitrates. The proteins of decaying plant and animal debris follow the course indicated below, each stage being accomplished by the activities of certain types of bacteria. In the first stage soil fungi are also active.

Bacteria and Moulds.	*Nitrosomonas, etc.*	*Nitrobacter.*
Protein ——→	Ammonium salts ——→	Nitrites ——→ Nitrates.

Other types, notably species of *Azotobacter* and *Clostridium* and the bacteria—*e.g.*, species of *Rhizobium*—that give rise to nodules in the roots of leguminous plants, are capable of "fixing" atmospheric nitrogen directly. In the first two cases the nitrogen becomes a constituent of the bacterial protein and is released only upon the death and decomposition of the organisms. The nodule bacteria enter and develop in the cortex of the plant root, where they stimulate rapid increase in plant cells. They utilise carbohydrates supplied by the plant, and in return the latter absorbs nitrogenous food material resulting from the bacterial activities, which include the fixation of atmospheric nitrogen. The high nitrogen content of leguminous plants and other factors have led to the adoption of the cultivation of certain legumes like clover, vetch, alfalfa, etc., in the rotation of crops now generally practised. Cultures of nodule organisms are now produced commercially for the

inoculation of leguminous seeds, although the specific organisms are often already present in the soil.

Under soil conditions of insufficient aeration and excessive organic matter, some of the valuable nitrates may be wasted through the activities of denitrifying bacteria, converting nitrates to nitrites or to free nitrogen. The following diagram, adapted from Russell (1923), illustrates the foregoing biological activities in relation to the nitrogen cycle:



NITROGEN CYCLE IN NATURE.

NOTE.—The diagram is reproduced by permission of Longmans, Green and Co., Ltd., from Sir John Russell's book, " Micro-organisms of the Soil."

The decomposition of the cellulose walls of dead plant tissues is, under aerobic conditions, carried out chiefly by

fungi, though certain types of bacteria participate. Under anaerobic conditions, as in water-logged soil, anaerobic bacteria are responsible for this breakdown, methane being a notable end product. Other bacteria decompose the starch, fats, pectins and other constituents. The carbon dioxide and organic acids formed as a result of such decompositions react with the calcium carbonate, potassium silicate, calcium phosphate and similar mineral substances of the soil. In excessively acid and air-free soils, as in the soil of bogs and moors, very little microbiological decomposition occurs. Hence dead plant remains accumulate with the formation of peat. Much liming is required to make strongly acid soil suitable for cropping.

The activities of "iron" and "sulphur" bacteria have already been mentioned (Chapter III.), but it should be noted that *Thiobacillus thiooxidans*, an autotrophic bacterium, oxidises sulphur and thiosulphate to sulphuric acid. Hence insoluble phosphates, if present, may be converted into a soluble form by its action (Waksman, 1931).

Of much interest is the effect of partial sterilisation on soil, effected either by heat or by adding antiseptics such as carbon bisulphide, formalin and toluol. When soil is so treated, its fertility is considerably increased. This phenomenon, mentioned on page 163 in another connection, has stimulated much research, perhaps the most notable being the work carried out at Rothamsted. Thus Russell and Hutchinson (1912-13) came to the conclusion that the increased fertility was due to the increase in available plant food resulting from the rapid development of bacteria, the latter being permitted since the predatory enemies of bacteria—namely, certain of the protozoa—are killed by the treatment. Other Rothamsted workers showed that the protozoan and bacterial population of the soil undergo fluctuations of reciprocal nature, and they produced additional evidence which supported Russell's theory. It is probable that in normal soils many other factors are involved, as, for example, the fungal flora, the change in the physical and chemical condition and particularly the available organic matter content of the soil (see Waksman, 1931). It is certain, however, that partial sterilisation results in a pronounced increase in the number of bacteria and amount of nitrate nitrogen (Burgess, 1929). Treatment of greenhouse

soils by steam and dry heat has already been mentioned (see Bewley, 1929).

Many bacteria capable of causing disease in plants—*e.g.*, soft rots—are normal inhabitants of soil.

Actinomycetes are important soil organisms, and in most agricultural soils form a high percentage of the colonies which develop on agar plates. These organisms are particularly resistant to drying, and are hence commonly found in sandy soil, dry straw, and so forth; they are not tolerant of acid conditions. Actinomycetes assist in the decomposition of most of the organic debris. The smell of freshly ploughed soil is probably largely attributable to actinomycetes.

Soil bacteria and actinomycetes may be readily demonstrated by the plate method. A good medium for enumerating these organisms is Thornton's medium (see Chapter VI.).

Thornton and Gray (1934) have shown that the plate method reveals only a fraction of the bacterial flora of the soil. By staining films of soil previously mixed with a known suspension of indigo particles and ascertaining microscopically the ratio of bacteria to indigo, they found that the numbers of bacteria in an average soil range from 1,000 to 4,000 million per gramme.

Soil Fungi.—These play a prominent part in the decomposition of proteins and vegetable matter and particularly cellulosic compounds.

Fungi in the soil are more difficult to enumerate than the bacteria, for they exist both as spores and mycelium. " Plating " methods emphasise the former, and fail to do justice to the latter, which is the active, and therefore more important, stage. A certain amount of information may be obtained by direct microscopic examination of soil films, and by examining the mycelium emanating from small particles of soil placed on agar media. The plating method, however, remains the most generally useful method of studying the fungal flora.

The saprophytes and facultative saprophytes—*i.e.*, organisms capable of either a parasitic or saprophytic existence—which form the bulk of the organisms present, come up readily on agar, the number of colonies per gramme of soil being often between 10,000 and 100,000. In order that these may not be swamped by the much larger numbers of bacteria, it is usual to suppress the latter by acidifying the medium slightly.

Czapek's (Dox's) agar, with the addition of 0·5 ml. of 12·5 per cent. lactic acid solution to each 100 ml. of medium at the time of plating, gives good results.

All the common moulds responsible for industrial troubles will be found in soil, and this is not surprising, since dust is the usual source of mould infections. *Aspergillus, Penicillium, Mucor* and related genera, *Cladosporium* and *Trichoderma* are representative types commonly found. These moulds are very important agents in humus formation, especially those that possess active cellulose decomposing power—*e.g., Aspergillus fumigatus, Cladosporium herbarum, Trichoderma lignorum,* and many species of *Penicillium.* These and other fungi break up the cellulose and hemi-celluloses of dead plant tissues and thus prevent the accumulation of vegetable debris.

In addition to the saprophytic fungi, there are two other groups of fungi important in soil. One is the soil-inhabiting fungi capable of causing plant diseases. Some of these, such as species of *Helminthosporium* and *Fusarium* causing foot-rots in cereals, may show themselves on laboratory media, but others are not detected by plating methods.

The other group consists of those fungi that grow in association with plant roots, the mycorrhizal fungi (Rayner, 1927). Many plants, notably the orchids, members of the *Ericaceæ* (heath family), and coniferous forest trees, habitually show a symbiotic relation between roots and mycorrhizal fungi, whose existence is sometimes essential for normal growth. The exact rôle played by mycorrhiza in plant nutrition is still largely unknown.

For further information on soil microbiology see Russell (1923) and Waksman (1931).

Miscellaneous.

Silage may be roughly described as pickled fodder for cattle. Green fodder, instead of being dried (as in the case of hay), may be preserved by stacking loosely in such a way as to encourage a lactic fermentation and consequent acidification of the whole mass that suppresses putrefactive bacteria. If conditions are too anaerobic, butyric organisms abound and the silage acquires an objectionable odour; too much aeration, on the other hand, leads to drying off, with mould develop-

ment and consequent mustiness. Material rich in nitrogenous matter may not develop acidity sufficiently quickly to stop putrefaction; this may be remedied by the addition of carbohydrate material—*e.g.*, molasses—or by the addition of mineral acids (A.I.V. process).

Artificial Farmyard Manure.—Various methods are advocated for compost making, or rapid humification of cellulosic material. In general, the essentials are free access of air, maintenance of sufficient humidity and provision of extra nitrogenous material—*e.g.*, ammonium sulphate, or cyanamide; the former is apt to make the material too acid. The organisms concerned are mould fungi and certain aerobic cellulose-decomposing bacteria.

Spontaneous Heating.—Hay and other plant materials, if stacked damp, develop heat which may lead to charring, or even to spontaneous ignition. Thermophilic bacteria may raise the temperature as high as 70° C., but any further rise of temperature is due to chemical oxidation.

Health of Labourers and Farm Animals.—The influence of micro-organisms on the health of live stock, transport animals and farm workers (see Chapter XII.) should not be forgotten as one of the economic factors affecting agriculture.

REFERENCES

ARTHUR, J. C. (1929): The Plant Rusts. New York.
BEWLEY, W. F. (1929): J. Min. Agric., 36, 623.
BROOKS, F. T. (1929): Plant Diseases. London.
—— (1935): Brit. Assoc. Meeting, Norwich; Presidential address to Section K.
BURGESS, R. (1929): Zentr. f. Bakt., II Abt., 78, 497.
BUTLER, E. J. (1918): Fungi and Disease in Plants. Calcutta.
ELLIOTT, C. (1930): Manual of Bacterial Plant Pathogens. London.
GODFREY, G. H. (1936): Phytopath., 26, 246.
GROVE, W. B. (1913): The British Rust Fungi. Cambridge.
HEALD, F. D. (1933): Manual of Plant Diseases. New York.
LÖHNIS, F., AND FRED, E. B. (1923): Textbook of Agricultural Bacteriology. New York.
MARTIN, H. (1936): Scientific Principles of Plant Protection. London.
NOWELL, W. (1923): Diseases of Crop Plants in the Lesser Antilles. London.
OUDEMANS, C. A. (1919-1924): Enumeratio Systematica Fungorum, Vols. 1-4. The Hague.
RAYNER, M. C. (1927): Mycorrhiza. London.
RUSSELL, E. J., *et al.* (1923): Micro-organisms of the Soil. London.
—— AND HUTCHINSON, H. B. (1913): J. Agric. Sci., 5, 152.

SACCARDO, P. A. (1882-1931): Sylloge Fungorum, Vols. 1-25. Pavia.
SEYMOUR, A. B. (1929): Host Index of the Fungi of North America. Harvard University Press.
SMITH, E. F. (1920): Bacterial Diseases of Plants. Philadelphia.
SORAUER, P. (1928-32): Handbuch der Pflanzenkrankheiten, Vols. 2 and 3. Berlin.
STANLEY, W. M. (1935): Science, **81**, 644.
—— AND LORING, H. S. (1936): *Ibid.*, **83**, 85.
STEVENS, F. L. (1913): The Fungi which cause Plant Disease. New York.
THORNTON, H. G., AND GRAY, P. H. H. (1934): Proc. Roy. Soc. London, B**115**, 522.
TURNBULL, J. (1934): J. Min. Agric., **40**, 1040. (1935), *Ibid.*, **42**, 865.
WAKSMAN, S. A. (1932): Principles of Soil Microbiology. London.

CHAPTER XIV

MISCELLANEOUS

Timber Decay.

WOOD is subject to attack by various fungi, but serious decay is usually attributable to the group *Basidiomycetes*. Certain common mould fungi are often the cause of surface discolorations, which may sometimes be removed by brushing or planing.

Of the basidiomycetes, some attack only the cellulose, whilst others utilise the lignified tissues, giving rise to " white rots." The fungi may occur on living or freshly felled timber, or on damp structural timber, as in the case of the dreaded " dry rot " caused by *Merulius lachrymans*. Timber affected by true dry-rot is light in weight, and shows a characteristic cracking into cubical blocks; in advanced stages of the decay the wood crumbles to dust at the slightest pressure. The fungus manifests itself as a whitish felt of mycelium, as string-like strands of interwoven hyphæ (rhizomorphs), or as reddish-brown, flat or bracket-like fructifications that give rise to enormous numbers of spores. Other harmful fungi on structural timber are *Poria vaporaria, Coniophora cerebella* and *Paxillus panuoides*—the two last occurring only under very damp conditions.

The most effective safeguard against wood-rotting fungi is to keep the wood dry; hence free access of air is essential to the successful seasoning of timber. Curiously enough, the opposite extreme—total immersion in water—is also practised occasionally—*e.g.*, logs of teak in Burma are weighted down below the surface of a river, the object being to secure conditions so anaerobic that neither fungi nor insects will be active.

Wood to be used for structural purposes should be thoroughly seasoned. The old method of log seasoning has been largely replaced by quicker modern methods involving steam heating. It has been claimed, however, that such methods—which destroy the cell enzymes and thus prevent the natural removal of

starch from the sap wood that occurs during log conditioning —render the timber more liable to fungal and insect attack (Wilson, 1933).

Timber that is to be used for outdoor work, where it is bound to become damp at times, must be protected by an antiseptic. Creosote is usually the cheapest of the effective substances for such work, its disadvantages being its pronounced odour and its tendency to volatilise. Proprietary substances made from tar oils are also efficient, although more costly. Other substances used for protection against fungi and insects are fluorides and silicofluorides, and salts of zinc, mercury, copper and arsenic, or combinations of these. The protection given by many of the water-soluble compounds may be lost owing to the leaching action of rain, and various suggestions have been put forward for producing by precipitation less soluble compounds within the wood.

The commonest methods of applying antiseptics to timber are painting or soaking (which gives little penetration), and pressure treatments (positive or negative pressure, or a combination of both); whatever the method, the antiseptic should preferably be applied hot. One effect of the increased consumption of treated timber is that the cheap porous timbers have increased in value, since they lend themselves more readily to impregnation with preservatives than do the heavier and more closely textured woods. When comparing the toxicity of wood preservatives, it is essential that work on laboratory media should be supplemented by tests on wood blocks and on timber exposed to actual weathering conditions. For further information see Thaysen and Bunker (1927), Cartwright and Findlay (1933), Findlay (1933), and Richardson (1937).

Wood Pulp and Paper.

Wood pulp is also subject to fungal attack, principally by staining moulds. According to Kress (1925), sodium fluoride and borax have been found to be among the most useful antiseptics for its protection. The moulds causing the spotting of paper have been investigated by Sée (1919). A bibliography of the microbiology of wood pulp and paper is given by Schulze (1932).

Rubber.

Rubber latex coagulates spontaneously through the development of acid-forming bacteria. For commercial purposes, however, chemical methods of coagulation with acids are more rapid and convenient. It is now a common practice to transport the rubber overseas to the factories in the form of latex, rendering it alkaline with ammonia in order to prevent coagulation during transport.

The principal interest of micro-organisms in connection with rubber is the danger of mould development on crêpe and other forms of rubber (Fullerton, 1929). Various species of *Penicillium*, *Aspergillus*, *Fusarium*, and even *Actinomyces* have been reported as causing deterioration of damp rubber in storage. Bacteria may also give rise to deterioration if the rubber is wet. In addition to causing a loss in weight due to gas evolution, micro-organisms may give rise to bubbles in the coagulum, to a condition of " rustiness " produced by decomposition of the serum substances which exude from the machined sheets, and to light coloured spots on crêpe rubber.

Smoking of sheet rubber gives some slight protection against fungi (O'Brien, 1927). For more complete protection various antiseptics have been recommended. Paranitrophenol is efficient, and may be used for smoked sheet, but if used for crêpe rubber it is liable to lead to yellow staining of certain coloured goods and wrapping paper. Sodium trichlorphenate, chinosol, and salicylanilide are other substances that have given good results.

Leather.

Micro-organisms affect the leather industry at various stages. Large numbers of hides and skins have to be destroyed following outbreaks of diseases like anthrax and foot-and-mouth disease. Lesions produced by ringworm fungi constitute imperfections in the raw material, though a minor source of loss compared with the damage done by warble fly. For the protection of workers against anthrax, disinfection of certain types of imported hide is compulsory in many countries (Robertson, 1931).

After removal from the animal, hides are dried, or are " pickled " in salt, in order to check the development of putre-

factive bacteria. Incompletely dried hides may be rendered useless. Salting suppresses putrefaction, but a number of halophilic bacteria and moulds still develop, and some of these lead to the formation of coloured stains—mainly red and violet (Bergmann, 1930). Such organisms may be suppressed by acidifying the pickling liquor, or by adding antiseptics such as paranitrophenol or sodium silicofluoride (Stuart and Frey, 1934; Robertson *et al.*, 1934).

During the soaking of hides preparatory to liming, proteolytic bacteria develop. Sheep skins often have the hair removed by a sweating process, in which bacteria are the active agents in loosening the hair. It is doubtful whether bacteria take much part in the liming process, though differing opinions have been expressed on this point. Probably enzyme action persists, but multiplication is checked by the alkaline reaction.

Soft leathers often receive a treatment in puer or bate liquors to render them pliable. Bacteria are supplied by inoculation with dog, hen, or pigeon dung, or with some proprietary solution; the process is usually carried out at 37° C. Attempts have been made at various times to substitute pure cultures—*Bac. subtilis*, *Bac. erodiens*, etc.—for the crude inoculation. Where drenching is practised, bacterial fermentation of bran gives rise to organic acids that protect wet skins against further putrefactive influence. Animal enzymes are now commonly employed in de-hairing processes; mould enzymes—*e.g.*, *Aspergillus oryzæ*—have also been advocated for the purpose (Berlinger, 1929).

Certain mould fungi, notably *Asp. niger*, are very tolerant of tannins, and often develop in tanning liquors. Among the antiseptics found useful for their suppression is beta-naphthol. This substance has also been recommended, as has paranitrophenol, for preventing the development of light-coloured, dye-resisting spots due to mould growth on chrome-tanned leathers.

For further information on leather microbiology see McLaughlin and Rockwell (1922) and Pickard (1923).

Paints and Building Material.

The so-called water paints, consisting or a gelatin or casein base in which the pigments and varnishes are emulsified, are

liable to develop mould or bacterial attack in storage, unless they are protected by the incorporation of a suitable antiseptic.

Far more difficult to prevent is the development of mould on painted surfaces subject to damp. This occurs with oil paints and enamels as well as with water paints. Zinc oxide pigments or certain added antiseptics exert some inhibiting action, but usually the only permanent cure is to remove the cause of dampness.

The mould fungi that are most troublesome are dark-coloured fungi imperfecti of the *Cladosporium* type. *Aspergillus niger*, *Asp. flavus*, and *Penicillium* spp. are frequent; *Phoma pigmentivora* causes red stains; and *Pullularia* (*Dematium*) *pullulans* is not uncommon on oil paints and varnishes. Moulds also occur on plaster walls, lime-washed or distempered. Magnesium silicofluoride has been successfully applied in such cases, but its acidity is liable to affect certain coloured washes.

Much of the humus-like or " soot " blackening that building materials undergo on weathering is undoubtedly biological in origin. Whether bacteria and moulds are active in the decay of stone is still an open question (see McLachlan, 1939).

Moulds and Arsenic Compounds.

Arsenic, although so poisonous to insects and animals, is not as a rule very toxic to fungi. Certain moulds, notably *Scopulariopsis brevicaulis* (=*Penicillium brevicaule*), when acting on a substrate containing traces of arsenic compounds, produce a volatile substance (trimethylarsine) which has a characteristic odour resembling that of garlic (Challenger, 1933). The liberation of this poisonous gas through the mildewing of plaster walls, or of wall-paper dyed with green dyes based on arsenic compounds, has not infrequently led to illness and even to death.

Tea, Coffee, Cocoa, Indigo, and Tobacco.

In the preparation of all the above products there is a " fermentation " stage, during which—as in the preparation of silage—plant enzymes, micro-organisms, and oxidation processes all play a part.

Tea.—Yeasts and bacteria occur during the curing process,

but are probably of little importance. The most important changes are brought about by plant enzymes, and by a mould which converts the tannic acid of the tea to gallic acid. If the fermentation is too prolonged or moisture is excessive, slime-forming bacteria develop, resulting in damage to the product (Marshall, 1921). Recent work in India indicates that only certain specific organisms are capable of developing in rolled leaf and causing loss of quality (Indian Tea Association, 1936).

Coffee.—Little work appears to have been done on coffee fermentation, but a patent has been taken out for a process of improving the flavour of coffee by encouraging the growth of certain fungi—*e.g.*, *Aspergillus ochraceus*—which occur naturally on the berries (Robison, 1919).

Cocoa.—Cocoa beans are fermented mainly to facilitate the removal of the pulp before drying. During this process changes in colour, aroma and flavour take place. An essential feature appears to be the development of yeasts; acetic bacteria that occur are probably of less importance, and mould fungi are definitely undesirable (Knapp, 1937).

In some cocoa-growing districts the fermentation is carried out in large wooden " sweat boxes "; in others the heaps are simply piled on the ground. In the latter method there is less drainage, and mould growth is liable to occur. Too rapid drying, on the other hand, is bad for the quality of the bean. Attempts have been made to replace fermentation by a heat treatment sufficient to kill the beans, but allowing enzyme action to continue throughout the early stages of drying. For a fuller account reference may be made to Briton-Jones (1934).

Indigo.—Indigo fermentation is now of less importance, since synthetic indigo has almost entirely superseded the natural product. The conversion of indican in the old indigo vat process was due to bacteria.

Tobacco.—After the leaves are dried, tobacco is exposed to controlled moist and warm conditions in order to secure desirable changes in colour and flavour. Bacteria and moulds develop during this period, and are considered by some to be essential to the process. However this may be, they cannot fail to affect the final product. Cigars and cigarettes often develop moulds in storage, *Aspergillus niger*, *Asp. fumigatus*, and species of *Penicillium* being of common occurrence.

Extraction of Vegetable Oils.

As a substitute for high pressure crushing of oil seeds, extraction by bacterial breakdown of the cell tissues has been proposed. Beckman (1930) describes a process using *Lactobacillus delbrückii*, at 50° C., for extracting the oil from dried copra. Further work seems desirable to find out the organism best suited to this type of work.

The Use of Micro-organisms for Chemical Analysis.

For the diagnosis of bacteria, and to a lesser extent that of fungi, the ability to ferment certain sugars has proved a very useful criterion. Conversely, when the fermentation reactions are known, the organisms can be employed as agents in analysis. Some yeasts, for example, will ferment lactose, most ferment dextrose, and some will not ferment maltose or saccharose; the use of suitable yeasts affords a method of separating these sugars (McLachlan, 1928; Gardner, 1939).

The production of a volatile arsenic compound by certain fungi has been referred to above (Challenger, 1933). This compound has a characteristic odour which becomes perceptible when minute traces of arsenic compounds are acted upon, and affords a very delicate test for arsenic.

For the estimation of available potassium and phosphorus in soils, the use of certain moulds (*Aspergillus niger*, *Cunninghamiella* sp., etc.) presents advantages over the purely chemical methods hitherto used (Smith and Dryburgh, 1934).

Future Developments in Economic Microbiology.

One encouraging feature of modern science is that the latent antagonism that used to exist between chemists and biologists is now passing away. New advances in microbiology are gained by the co-operation of both forces, and by the coming into existence of a new type of expert who has a foot in each camp.

As a result of such co-operation there has been a rapid increase of recent years in our knowledge of fungicides and bactericides. The specificity of such substances is very great, and possibilities already touched but still to be exploited include bactericides harmless to animals (as internal antiseptics), fungicides harmless to plants (for plant protection),

and antiseptics which permit a pure culture fermentation but inhibit infections. The last possibility, permitting open vessels to be used, and avoiding the expense of sterilisation and aseptic precautions, has long been foreshadowed by the protective action of hop antiseptics in brewing and by the "acclimatisation" (or possibly selection) of yeast races to withstand sodium fluoride. Of internal antiseptics, sulphanilamide and certain of its compounds have given good results with human diseases associated with pneumococci, staphylococci and streptococci (Buttle, 1939).

The influence of environmental factors—particularly reaction and nutrient supply—on the products of metabolism has been emphasised in Chapter VII. Work on these lines should continue to produce important results. Other possible advances in fermentation work may be brought about by the more extensive use of mixed cultures, and of continuous methods of fermentation. Mould fermentations are at present usually carried out in shallow trays; their more extensive use will perhaps be in tanks through which air is blown, or in a trickling filter as used in vinegar manufacture.

The utilisation of waste products is by no means as complete as it might be. Of the three main plant products, starch is already exploited adequately, cellulose only partially, and hemicelluloses hardly at all. Dairy waste, sewage, and various animal products are still incompletely utilised.

Other possible developments are difficult to prophesy. Storage problems will continue to be brought under control. Some further enzyme preparations derived from bacteria or fungi will no doubt find use in various industries. The production of odours, flavours or dyestuffs from micro-organisms is frequently suggested, but it is very doubtful whether work on these lines will lead to results capable of commercial exploitation. The synthetic polysaccharide processes touched upon in Chapter VII. may be developed for large-scale production of gums and parchment-like materials (Sanborn, 1936).

The organisation of economic microbiology is very incomplete. Central institutes exist for the study of medical bacteriology and of plant pathology, but there is no centralisation of research and advisory work in connection with the numerous problems, large and small, that affect industry. Most of these problems are due to common "soil organisms"

which are surprisingly uniform throughout the world, and the principles by which the problems may be solved are also surprisingly similar whatever the industry concerned. The establishment of a central clearing house for pooling and distribution of the results obtained by food, fermentation, textile and other microbiologists would be a very desirable step.

REFERENCES

BECKMAN, J. W. (1930): Ind. Eng. Chem., **22**, 118.

BERGMANN, M. (1930): Amer. Leather Chem. Assn., **25**, 256.

BERLINGER, C. (1929): Cuir Tech., **18**, 132.

BRITON-JONES, H. R. (1935): The Diseases and Curing of Cacao. London.

BUTTLE, G. A. H. (1939): Brit. Med. J., August, 269.

CARTWRIGHT, K. ST. G., AND FINDLAY, W. P. K. (1933): Forest Prod. Res. Bull. No. 1.

CHALLENGER, F. (1933): Ind. Chemist, **9**, 134.

CHAPMAN, CHASTON, *et al.* (1923): J. Soc. Chem. Ind., **42**, 169T.

CUNNINGHAM, A. (1934): Practical Bacteriology.

FINDLAY, W. P. K. (1933): Sci. Progress, **28**, 61.

FOWLER, A. J. (1934): Introduction to the Biochemistry of Nitrogen Conservation.

FULLERTON, R. G. (1929): Rev. Appl. Myc., **8**, 524.

GAIGER, S. H., AND DAVIES, G. O.: Veterinary Pathology and Bacteriology.

GARDNER, H. A., HART, L. P., AND SWARD, G. G. (1933): Amer. Paint and Varnish Mfrs. Assn. Cir. 442. (See also *ibid.*, Cirs. 448, 464, and 475.)

GARDNER, R. (1939): Analyst, **64**, 103.

HARDING, V. J., AND NICHOLSON, T. F. (1933): Biochem. J., **27**, 1082.

INDIAN TEA ASSOCIATION (1936): Tocklai Exp. Sta. Ann. Report, 1935.

JENSON, P. O. (Arup.) (1931): Dairy Bacteriology.

JORGENSEN, A. (1939): Micro-organisms and Fermentation.

KNAPP, A. W. (1937): Cacao Fermentation. London.

KRESS, O., *et al.* (1925): U.S.D.A. Bull. 1298.

McLACHLAN, T. (1928): Analyst, **53**, 583.

—— (1939): abst. Rev. Appl. Myc., **18**, 333.

McLAUGHLIN, G. B., AND ROCKWELL, G. E. (1922): J. Amer. Leather Chem. Assn., **17**, 325.

MARSHALL, C. E. (1921): Microbiology. London.

O'BRIEN, T. E. H. (1927): abst. Rev. Appl. Myc., **6**, 314.

PICKARD, C. E. (1924): Chem. and Ind., **43**, 476.

PLEASS, W. B. (1935): J. Soc. Leather Trades Chem., **19**, 4.

RICHARDSON, N. A. (1937): Chem. and Ind., **56**, 1148.

ROBERTSON, M. E. (1931): Amer. Leather Chem. Assn., **26**, 120.

—— MARRIOTT, R. H., AND HUMPHREYS, F. E. (1934): J. Soc. Chem. Ind., **53**, 83T.

ROBISON, F. W. (1919): U.S. Pat. 1,313,209.

SALLE, A. J. (1939): Fundamental Principles of Bacteriology.

SANBORN, J. R. (1936): Ind. Eng. Chem., **28**, 1189.

SCHULZE, B. (1932): Papierfabr., **30**, 486, 497.

SÉE, P. (1919): Les Maladies de Papier Piqué. Paris.
SMITH, A. M., AND DRYBURGH, A. (1934): J. Soc. Chem. Ind., 53, 250T.
SMITH, G. (1938): Introduction to Industrial Mycology.
STUART, L. S., AND FREY, R. W. (1934): J. Amer. Leather Chem. Assn., 29, 118.
TANNER, F. W. (1937): Bacteriology. New York.
THAYSEN, A. C., AND BUNKER, H. J. (1927): Microbiology of Celluloses, etc. London.
WEISE, K. (1934): Farben Ztg., 39, 412.
WILSON, S. E. (1933): Ann. Appl. Biol., 20, 661.

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